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A LITERATURE SURVEY ORIENTED TOWARDS ADVERSE ENVIRONMENTAL EFFECTS RESULTANT FROM THE USE OF AZO COMPOUNDS, BROMINATED HYDROCARBONS, EDTA, FORMALDEHYDE RESINS, AND O-NITROCHLOROBENZENE

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FINAL REPORT

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF TOXIC SUBSTANCES
WASHINGTON, D.C. 20460

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SUMMARY AND CONCLUSION AS TO DEGREE OF HAZARD

A very large number of azo dyes is in production throughout the world, but amounts made vary over a wide range and change considerably from year to year according to the whims of fashion and design. Biological studies do not seem to have been done on the majority of these. Other than an occasional case of skin allergy to a clothing dye, there doesn't seem to be any need for concern about the azo dyes used in cloth, paints, plastics, inks, etc. The dyes used in food, drugs, and cosmetics vary from one country to another, each of the latter seeming to have a different set of standards for rating studies for toxicity in laboratory animals. Currently undergoing investigation is the neglected area of teratogenic effects. Because of the wide range of foods a particular dye may be found in, it has proven rather difficult to extrapolate "no adverse effect" levels in animals to humans and then set a daily overall consumption limit.

Metabolism studies of the dyes have indicated that many are cleaved only by the intestinal flora, a rather variable factor even in highly inbred populations of laboratory animals. Both oil and water soluble dyes seem able to pass through the intestinal wall in both directions, thus complicating these studies.

Teratogenetic studies have been done most frequently on Trypan Blue and its related biological stains. Trypan Blue has been shown to be a variably complex mixture, neither in part nor in whole consistently teratogenic. The related dyes show a lesser degree of teratogenicity, the studies complicated by mislabeling by manufacturers.

Carcinogenicity of azo dyes in humans has not been demonstrated, possible occupational cases being compromised by concurrent exposure to carcinogenic starting materials. Many studies have been conducted on derivatives of ring- and N-methylated 4-aminoazobenzene. Rats have developed cancers of the liver when fed some of these for two months, usually in a low-protein, low-riboflavine diet. Other laboratory animals show much less to no susceptibility to these hepatocarcinogens. Researchers have been unable to unravel the sequence of events leading to the initiation of tumor growth, there being no correlations of internal physical or chemical changes and relative carcinogenicities to guide them.

Studies on the flour additive azodicarbonamide have shown that it is completely altered to another compound in the baking process, biurea, which was not toxic at the level involved.

Considering the number of azo compounds in distribution and the uses to which they are being put, the lack of hazard is remarkable. The greatest danger appears to be from the ingestion of unauthorized food dyes. Adequate supervision of imports and thorough testing of new dyes should protect the consumer.

AZO COMPOUNDS

I. PROPERTIES

This report is confined to compounds having the linkage C-N=N-C. No attempt was made to compile a list of melting points for the azo dyes or the azo compounds used in cancer research because: most commercial products, even dyes authorized for human consumption, are mixtures of different compounds or isomers; structural features such as sulfonic acid salts and great complexity tend to induce decomposition prior to melting; the heating process could induce changes in the isomerism about the azo linkage(s), or tautomerism about highly electropositive/electronegative functional groups prior to melting, so that any melting point might not be for the original structure. In general the azo dyes are not water soluble unless they have at least one sulfonic acid group ($-SO_3H$), and too many of these makes the compound insoluble in organic solvents. All of the food, drug, and cosmetic dyes are water soluble, but nearly insoluble in most organics. Of the FD&C's, Red No. 2 has a solubility in glycerine or propylene glycol 1.5 or 0.083 times, respectively, that in water; for Red No. 4 the comparable figures are 0.55 or 0.16; for Red No. 40, 0.14 or 0.07; for Yellow No. 5, 1.6 or 0.68; for Yellow No. 6, 0.63 or 0.11.

Azobenzene, the simplest azoaromatic, exists at room temperature in the trans form, mp 68°C, but can be isolated under colder conditions in the cis form, mp 71°C. The trans form has a specific gravity of 1.203 (20/4°), a vapor pressure of 1 mm Hg at 103.5°C, a bp of 300°C, and is soluble in organics.

2,2'-Azobisisobutyronitrile, NC $C(CH_3)_2N$ = NC $(CH_3)_2CN$, melts at 105°C with decomposition, is soluble in organics, insoluble in water.

Azodicarbonamide, $H_2NC(O)N = NC(O)NH_2$, melts at 180-4°C with decomposition, is soluble in dimethyl sulfoxide, insoluble in the common organics and in water.

Sawicki (1957) studied the tautomerism of 73 aminoazobenzene compounds in a 50% alcoholic HCl solution by measuring the UV spectra, reporting values for pK_a and for the ratio of the intensities of the absorptions due to the proton being on one of the azo N's or on the amino N.

Gerson and Heilbronner (1962) did a similar study on p-N,N-dimethylaminoazobenzene and ten derivatives, reporting the values for mp, difference in pK_a between the parent and the derivative, and the absorption intensity ratio. They also studied the tautomerism of p,p'-bis(dimethylamino)azobenzene in a variety of acid-solvent systems (pp. 51-9 of the indicated reference).

Bershtein and Ginzburg (1972) have reviewed the literature through 1969 concerning the tautomerism of hydroxy- and aminoazo compounds.

In Section VI (Monitoring and Analysis) of this report are reviewed a number of papers which discuss the nature of the impurities in specific azo compounds.

II. PRODUCTION

Production of azo dyes and azo pigments in the United States has been increasing steadily since 1958 following a plateau from 1952-1958. Statistics for the dyes and pigments are given separately in the following tables.

Table 1. United States Production of Azo Dyes

1952	22.3[a]	1963	24.6
1953	25.9	1964	25.9
1954	24.1	1965	30.4
1955	27.3	1966	31.8
1956	23.2	1967	26.8
1957	23.2	1968	32.3
1958	18.2	1969	34.1
1959	23.6	1970	
1960	20	1971	37.3
1961	20.5	1972	41.8
1962	23.6		

a Units are in 1,000 metric tons

Table 2. United States Production of Azo Pigments

1961	8.82[a]	1967	12.2
1962	9.08	1968	12.5
1963	9.45	1969	14.1
1964	10.0	1970	12.9
1965	10.9	1971	13.9
1966	11.5		

a Units are in 1,000 metric tons

Production statistics on individual azo dyes and pigments for 1971 and 1972 (including imports for 1972) follow. As with the overall annual figures these have been taken from the United States Tariff Commission Reports. The dyes for which no figures were given therein (to protect the

few companies making them) are included in an overall compilation indicating the manufacturers. Many azo dyes have, very likely, been excluded because it was not possible to identify them as such, the available volumes of the only reference work *Colour Index* not covering the latest ten years.

Table 3. Production (1971, 1972) and Importation (1972) in the United States of Individual Azo Dyes and Pigments

		Acid Dyes[a]		
Yellow	9	-[b]	-[c]	0.91[d]
	11	34.6	24.1	-
	17	258	214	-
	19	-	-	76
	23	142	127	5.1
	25	-	-	10.7
	34	35.4	N.R.	-
	36	87.2	74.5	1.95
	38	72.6	-	14.7
	40	154	45.4	-
	42	36.8	40	2.4
	44	7.72	N.R.	-
	54	17.3	50	-
	64	-	-	8.75
	65	N.R.	35.4	-
	70	-	-	0.30
	72	-	-	0.70
	76	23.6	17.3	-
	99	47.7	21.4	0.25
	121	-	-	0.45

	124	-	N.R.	-
	127	-	-	1.50
	128	-	-	1.02
	135	-	-	58.5
	136	-	-	0.50
	151	591	600	-
	159	267	213	-
Orange	7	254	224	0.20
	8	128.5	172	0.25
	10	123	131	-
	19	-	-	2.23
	24	425	332	-
	28	-	-	3.41
	33	-	-	5.16
	51	-	-	1.25
	60	86.3	110	-
	61	-	-	0.50
	64	21.8	-	-
	74	37.2	40.4	-
	92	-	-	0.68
	94	-	-	11.8
	102	-	-	0.90
Red	1	197	173	-
	4	46.3	51.3	-
	6	-	-	0.023
	14	51.3	N.R.	-

18	52.2	48.1	-
26	20.4	17.3	-
32	-	-	0.91
35	-	-	0.11
37	35.4	38.6	0.70
42	-	-	3.55
57	-	-	20.3
73	117	107	1.61
85	73.6	57.2	0.68
88	372	532	-
89	8.2	19.1	-
99	79	76.8	0.25
111	-	-	32.2
114	156	192	4.10
115	34.1	17.7	-
127	-	-	5.25
131	-	-	11.1
134	-	-	0.95
137	81.7	90.7	-
151	292	446	0.27
155	-	-	0.35
157	-	-	1.61
158	-	-	0.455
161	-	-	3.41
179	-	-	0.60
182	30.4	32.7	-
186	12.3	-	-

	240	-	-	0.10
	249	-	-	6.31
	251	-	-	5.91
	252	-	-	3.23
	257	-	-	10.9
	258	-	-	1.90
	259	-	-	1.31
	260	-	-	12.1
	261	-	-	1.05
	263	-	-	3.76
	266	55.9	122	22.8
	274	-	-	1.25
	282	-	-	1.80
	283	-	-	2.25
Violet	1	17.3	13.2	-
	3	39.6	55.9	-
	5	-	-	0.451
	7	43.6	61.8	-
	12	-	-	-
	14	-	-	1.80
	90	-	-	0.125
Blue	92	44.5	N.R.	-
	113	378	382	45.4
	118	40.4	33.6	-
	120	-	15.4	0.371
	151	-	-	1.72
	154	-	-	0.60

	156	–	–	2.16
	158/158A	45.9	55	–
	163	–	–	0.05
	183	–	–	0.326
	184	–	–	1.24
	187	–	–	5.06
	193	–	–	0.60
	199	–	–	0.125
	205	–	–	2.75
Green	20	23.6	27.7	–
	60	–	–	0.20
	68	–	–	2.18
Brown	14	394	338	–
	33	–	–	18.1
	83	–	–	8.74
	85	–	–	5.50
	127	–	–	5.00
	224	–	–	5.52
	226	–	–	0.266
	227	–	–	0.651
	235	–	–	22.8
	239	–	–	24.6
	253	–	–	0.114
	264	–	–	4.67
Black	1	427	474	2.36
	24	–	24.5	1.28

	52	315	442	-
	76	-	-	1.50
	82	-	-	0.150
	84	-	-	0.97
	107	93.5	145	14.1
	108	-	-	0.60
	117	-	-	3.00
	128	-	-	0.80
	131	-	-	19.6
	132	-	-	19.1
	139	-	-	7.61
Azoic Diazo Component 4, base		81.3	89	-
		Basic Dyes		
Orange	1	139	N.R.	-
	2	224	203	0.274
	28	-	-	1.82
	29	-	-	0.227
	30	-	-	8.26
Red	18	-	-	0.227
	23	-	-	38.4
	24	-	-	0.341
	25	-	-	1.93
Brown	1	29.5	66.8	-
	4	218	218	0.05

Direct Dyes

Yellow	4	214	226	-
	8	-	-	0.65
	12	117	91	0.15
	27	-	-	2.01
	28	110	108	-
	29	19.5	14.5	-
	44	396	506	1.82
	50	221	229	-
	69	-	-	0.227
	84	346	328	-
	93	-	-	1.40
	95	-	-	0.85
	98	-	-	22.5
	109	-	-	0.57
	110	-	-	1.18
Orange	1	10	N.R.	-
	8	28.2	49.5	-
	26	26.8	31.8	-
	29	56.7	46.4	-
	34	49.1	50.9	-
	37	17.7	11.8	-
	39	88.6	104	-
	66	-	-	1.24
	72	180	148	-
	73	-	51.4	-
	81	N.R.	36.4	-

	102	117	189	-
	106	-	-	1.75
	107	-	-	17.5
Red	1	56.4	58.1	5.16
	2	113	92	-
	3	-	-	1.36
	4	18.6	30	-
	9	-	-	4.91
	10	4.55	N.R.	-
	11	-	-	0.60
	13	20.4	N.R.	-
	16	N.R.	60.5	-
	23	109	111	0.50
	24	181	164	-
	26	43.2	47.7	-
	28	48.1	79	2.27
	31	7.27	-	-
	37	49.1	48.6	-
	39	67.3	73.6	-
	62	-	-	2.04
	72	85.9	123	-
	75	9.1	7.72	1.68
	79	37.2	102	3.03
	80	274	305	1.02
	81	243	242	-
	83	106	114	3.00
	84	-	-	0.226

	89	-	-	2.94
	122	-	N.R.	-
	123	-	N.R.	-
	152	-	-	3.64
	173	-	-	1.00
	205	-	-	1.80
	207	-	-	4.08
	212	-	-	0.795
	218	-	-	0.91
Violet	7	-	N.R.	2.66
	9	87.6	68.6	-
	47	-	-	13.7
	48	-	-	9.03
	51	-	10.9	1.93
	93	-	-	9.10
	95	-	-	2.50
Blue	1	136	172	-
	2	580	466	-
	6	119	131	-
	8	64	106	-
	10	-	-	3.41
	15	94	108	-
	22	-	10.45	-
	24	-	N.R.	-
	25	22.7	29.5	0.455
	67	-	N.R.	-

	71	-	53.6	-
	76	53.2	30.9	-
	78	64	60.4	-
	80	286	256	-
	98	39	154	-
	112	-	-	0.136
	120/120A	-	66.3	30.6
	126	92	65.5	-
	149	-	-	2.73
	156	-	-	2.32
	158	-	-	24.3
	207	-	-	5.26
	211	-	-	2.70
	218	479	499	-
	225	-	-	2.16
	239	-	-	0.34
Green	1	109	106	-
	6	235	184	-
	26	-	-	2.50
	33	-	-	0.318
	51	-	-	6.07
	59	-	-	2.15
	67	-	-	6.61
	68	-	-	1.59
	69	-	-	1.10
	74	-	-	0.318

Brown	1A	-	N.R.	-
	2	120	117	-
	31	75.5	55	-
	74	30.9	30	-
	95	240	230	7.51
	111	-	13.6	-
	154	202	178	-
	200	-	-	8.52
Black	4	39.6	45	-
	9	20.9	-	-
	19	-	N.R.	-
	22	303	853	-
	38	2,400	3,050	-
	51	22.7	31.4	-
	62	-	-	2.08
	71	-	-	1.43
	80	304	370	-
	91	-	-	1.08
	112	-	-	1.34
	113	-	-	1.00
	114	-	-	3.06
	118	-	-	14.0
	122	-	-	0.85
		Disperse Dyes		
Yellow	3	1,127	1,278	0.125
	5	N.R.	34.1	8.85

	7	-	-	0.34
	23	527	372	1.77
	44	-	-	13.4
	50	-	-	2.00
Orange	1	-	-	0.20
	3	61.4	50.9	-
	5	100	22.7	2.81
	13	-	-	12.5
	17	120	59.1	-
	18	-	-	1.58
	20	-	-	14.0
	25	210	224	-
	30	-	-	5.61
	38	-	-	0.60
Red	1	139	119	-
	5	62.6	47.7	-
	13	-	N.R.	-
	17	97	70	-
	44	-	-	20.0
	46	-	-	4.85
	54	-	-	35.4
	65	105	105	-
	72	-	-	38.5
	73	-	-	129
Brown	1	-	-	2.41
	2	67.3	N.R.	1.92

Black	1	-	152	-
		FD&C Dyes		
Red No.	2	474	441	-
	4	N.R.	N.R.	-
	40	N.R.	N.R.	-
Yellow No.	5	561	504	-
	6	464	369	-
		D&C Dyes		
Orange No.	4	3.18	-	-
Red No.	6	4.08	N.R.	-
	7	-	-	-
	9	16.4	N.R.	-
	36	-	4.08	-
		Mordant Dyes		
Yellow	1	7.72	N.R.	-
	8	-	N.R.	-
	26	-	-	6.49
	30	-	-	0.114
Orange	3	-	-	2.95
	22	-	-	1.00
Red	5	-	-	0.91
	17	-	-	1.02
Blue	7	-	-	6.56

Green	29	-	-	0.10
	47	-	-	1.00
Brown	1	11.8	18.2	0.114
	21	-	-	1.59
	33	22.3	-	-
	40	-	N.R.	-
Black	11	248	200	16.1
	17	45.4	68.6	0.095
	79	-	-	2.73
		Reactive Dyes		
Yellow	4	-	-	0.20
	6	-	-	0.50
	11	-	-	1.00
	12	-	-	5.51
	18	-	-	0.20
Orange	3	-	-	0.25
	5	-	-	1.43
	7	-	-	2.65
Red	4	-	-	0.97
	6	-	-	1.85
	7	-	-	2.00
	9	-	-	0.90
	12	-	-	11.0
	13	-	-	7.36
	15	-	-	4.40

	16	-	-	4.31
	17	-	-	23.6
	19	-	-	3.50
	21	-	-	3.50
	22	-	-	2.90
	23	-	-	0.97
	24	-	-	0.33
Violet	3	-	-	7.70
	5	-	-	4.55
Blue	8	-	-	41.9
	10	-	-	34.2
	13	-	-	16.8
Brown	2	-	-	8.85
Black	4	-	-	6.56
		Solvent Dyes		
Yellow	2	10.0	N.R.	-
	14	261	269	0.625
	16	-	-	1.49
	19	-	-	1.03
	21	-	-	0.67
	25	-	-	3.17
	29	-	-	0.045
	32	-	-	0.075
	62	-	-	2.70
	63	-	-	1.90

	65	-	-	7.36
Orange	3	24.6	54.9	0.92
	5	-	-	0.30
	6	-	-	0.025
	7	34.5	N.R.	-
	9	-	-	0.115
	11	-	-	2.72
	41	-	-	2.40
	44	-	-	0.45
	45	-	-	0.025
Red	1	-	-	0.10
	3	-	-	0.52
	7	-	-	1.17
	9	-	-	0.50
	12	-	-	0.015
	16	-	-	0.065
	18	-	-	7.56
	19	-	-	0.50
	24	-	-	0.33
	26	134	119	-
	27	-	-	0.25
	30	-	-	4.84
	36	-	-	0.065
	90	-	-	5.01
	91	-	-	4.93
	92	-	-	0.40
	109	-	-	7.95

Violet	1	-	-	0.025
	24	-	-	0.24
Blue	53	-	-	0.015
Brown	1	-	-	0.45
	12	10.4	15	-
	28	-	-	2.0
	34	-	-	1.67
	35	-	-	0.025
	37	-	-	0.57
Black	1	-	-	0.035
	2	-	-	2.52
	3	-	-	15.1
	6	-	-	0.33
		Pigments[e]		
Yellow	1	784		20.8
	3	180		15.4
	12	2,550(+1,486)		45.1
	13	(See 17)		10.9
	14	1,033(+1,385)		12.2
	16	-		32.1
	17	259 (Including No. 13, others)		1.04
	49	-		1.25
	55	-		0.025
	73	-		4.55
	74	243		-

	81	-	19.8
	83	-	23.0
Orange	1	-	0.25
	5	182	27.8
	13	65.4	4.06
	16	153	-
	31	-	32.6
Red	1	85.8	-
	2	28.2	-
	3	738(+649)	22.3
	4	140	-
	5	38.6	1.50
	7	-	1.55
	9	-	29.6
	10	-	0.225
	14	-	3.0
	17	30.4	-
	22	60.8	-
	23	88	-
	38	60.4	0.10
	48	1,230(+1,090)	6.64
	49(Total)	2,640(+1,840)	
	51	-	0.075
	52	790	-
	53	1,100(+855)	26.6
	54	31.3	-

		57	465	15.7
		63	19.1	5.09
		68	-	2.0
		112	-	32.5
		119	-	0.045
		144	-	25.0
		144(<90%)	-	135
		146	-	8.50
		151	-	4.50
Blue		25	87.6	-
Green		10	-	7.26
Brown		5	69.5	-
Pigment	*Red*	60))Lakes	126	-
Acid	*Red*	26)	88	-
Permanent (Toner)	*Red*	F4RH	-	2.5

a Listing is alphabetical by type of dye; within a type, order of color is that used by Colour Index and U.S. Tariff Commission Reports. Units are in metric tons.

b,c,d U.S. 1971, 1972, and imports, 1972, respectively; N.R. means Not Released; a dash for a year preceeded or followed by a year represented by a number or N.R. means that the dye was listed in the production figures section of the U.S. Tariff Commission Report, but no figure was given. Except for Acid Violet 12 dashes for both

years mean that the dye was not represented in the production figures section.

[e] Left column is U.S. 1971, right one is 1972 imports. Figure in parentheses is for the commercial forms.

There follows a copy of the listing of dyes whose production was reported in the 1971 U.S. Tariff Commission Report; sulfur and vat dyes were omitted from the end as these do not seem to contain azo linkages. Confirmed azo dyes have been indicated by a dot centered to the right of the dye's name. Production figures for those dyes marked with an asterisk are given in Table 3. The next table (5) is a listing of the azo dyes produced in 1971, but not in 1972. Then there is a table (6) of dyes produced in 1972, but not in 1971. Finally there is a table (7) of manufacturers, whose code names appeared beside the dye names. **Tables 4-7 are in the Appendix.**

In 1972 the U.S. Tariff Commission reported the following importation of azo compounds under the designation of Benzenoid Chemicals and Products: 4-aminoazobenzene or Solvent Yellow 1 (4.5 metric tons); 4-aminoazobenzene-disulfonic acid (23 metric tons); 4-aminoazobenzene-3,4'-disulfonic acid, monosodium salt (13.6 metric tons); 2-aminoazobenzene-4',5-disulfonic acid or Acid Yellow 9 (36.7 metric tons); azobenzene (58.6 metric tons); and 4,4'''-azobis(4-biphenylcarboxylic acid) or azo yellow acid (15.4 metric tons).

The Chemical Week Buyers Guide 1974 Edition offers for sale the following azo compounds which are not to be found in the U.S. Tariff Commission Reports under domestic production: 4-aminoazobenzene (Solvent Yellow 1) and its hydrochloride; azobenzene; 4,4'-azobis(N,N'-dimethylaniline); azosulfamide (also known as Prontosil S or Neoprontosil) and which has the chemical formula 2-(4-sulfonamidophenylazo)-3,6-disulfo-7-acetamido-naphthol-1, disodium salt; 2,6-diamino-3-phenylazopyridine hydrochloride; 4-hydroxyazobenzene (Solvent Yellow 7); and Methyl Red (Acid Red 2).

In the Cyclic Intermediates section of the U.S. Tariff Commission Reports are listed production figures for a few dyes, with manufacturers, plus an alphabetical listing of dyes and dye intermediates apparently lacking Colour Index names: Acid Yellow 9, 5.91 metric tons in 1971, American Cyanamid Co., Nyanza Inc., Toms River Chemical Corp.; Food Yellow 6, 141 and 207 metric tons in 1971 and 1972, respectively, Allied Chemical Corp., A. Cyanamid, duPont, Toms River; Solvent Yellow 3, 148 and 180 metric tons, Alliance Chemical, Inc., A. Cyanamid, duPont, GAF, Sterling Drug, Inc.

Table 7a. Domestic Manufacture in 1971 and 1972 of Azo Intermediates in the Dye Industry

8-acetamido-1-(4-acetamido-2-hydroxy-5-nitrophenylazo)-2-naphthol, 1972, Toms River;

3-[(2-acetamido-4-aminophenyl)azo]-1,5-naphthalenedisulfonic acid, 1972, Toms River;

5-amino-4,5'-dihydroxy-3,4'-[(2-methoxy-5-methyl-p-phenylene)bis(azo)]-di-2,7-naphthalenedisulfonic acid, 5'-benzenesulfonate, 1971, 1972, Toms River;

2-(2-amino-5-hydroxy-7-sulfo-1-naphthylazo)-5-nitrobenzoic acid, 1971, 1972, Toms River;

m-[(4-amino-3-methoxyphenyl)azo]benzenesulfonic acid, 1971, 1972, duPont, Toms River;

4-[(4-amino-5-methoxy-o-tolyl)azo]-4-hydroxy-2,7-naphthalenedisulfonic acid benzenesulfonate, 1971, 1972, Toms River;

3-[(4-amino-5-methoxy-o-tolyl)azo]-1,5-naphthalenedisulfonic acid, 1971, 1972, Toms River;

7-[(4-amino-5-methoxy-o-tolyl)azo]-1,3-naphthalenedisulfonic acid, 1971, 1972, Toms River;

2-(4-amino-1-naphthylazo)-4-(1,1,3,3-tetramethylbutyl)phenol, 1972, GAF;

m-[(p-aminophenyl)azo]benzenesulfonic acid, 1971 (duPont, Toms River), 1972, Toms River;

7-[(4-aminophenyl)azo]-1,3-naphthalenedisulfonic acid, 1971, 1972, Toms River;

5-amino-8-(phenylazo)-2-naphthol, 1971, 1972, Alliance;

8-amino-5-(phenylazo)-2-naphthol, 1971, 1972, Alliance;

4-[(p-aminophenyl)azo]-1-naphthylamine, 1971, 1972, Allied;

5-[(p-aminophenyl)azo]salicylic acid, 1971 (Toms River), 1972 (Baychem Corp., Toms River);

preceeding item, sodium salt, 1971, 1972, Allied;

m-(4-amino-3-tolylazo)-benzenesulfonic acid, 1971, 1972, Toms River;

3-[(4-amino-o-tolyl)azo]-1,5-naphthalenedisulfonic acid, 1971, 1972, Toms River;

7-[(4-amino-o-tolyl)-azo]-1,3-naphthalenedisulfonic acid, 1971, 1972, Toms River;

3-(o-anisylazo)-benzensulfonic acid, sodium salt, 1971, 1972, Allied;

4',4'''-azobis(4-biphenylcarboxylic acid), 1971, 1972, duPont, Toms River;

3-(4-N-benzylamino-N-methylphenylazo)-1,2,4-triazole, 1972, Toms River;

4,4'-bis-[(p-hydroxyphenyl)azo]-2,2'-stilbenedisulfonic acid or Direct Yellow 4, 1971, Toms River;

N-(2-chloroethyl)-4-(2-chloro-4-nitrophenylazo)-N-ethyl aniline, 1971, 1972, GAF;

N-[(5-chloro-2-methoxyphenyl)azo]sarcosine, 1971, 1972, Atlantic Chemical Corp.;

N-[(5-chloro-o-tolyl)azo]sarcosine, 1971, 1972, Alliance, Atlantic;

dibenzylazodicarboxylate, 1972, Kay-Fries Chemicals, Inc., Wilson & Co., Inc.;

2-(5,8-dichloro-1-hydroxy-2-naphthylazo)-1-phenol-4-sulfonamide, 1972, Toms River;

3-[(4'-N,N-diethylamino)phenylazo]-1H-1,2,4-triazole, 1971, 1972, Toms River;

4,5-dihydroxy-3-(p-sulfophenylazo)-2,7-naphthalenedisulfonic acid, trisodium salt, 1971, 1972, Eastman Kodak Co.;

N,N'-[(3,3'-dimethoxy-4,4'-biphenylene)bis(azo)]bis(N-methyltaurine), 1972, GAF;

4-(α,α-dimethylbenzyl)-2-phenylazophenol, 1971, Toms River;

N,N-dimethyl-p-phenylazoaniline, 1972, Eastman;

1-(3,5-dinitro-2-hydroxy-phenylazo)-2-naphthol, 1971, 1972, Toms River;

2-[N-ethyl-p-[(6-methoxy-2-benzothiazolyl)azo]anilino]ethanol, 1971, 1972, Toms River;

N-[7-hydroxy-8-[2-hydroxy-5-(methylsulfamoylphenyl)azo]-1-naphthyl]-acetamide, 1971, 1972, Toms River

6'-hydroxy-5'-[2-hydroxy-5-nitrophenyl)azo]-m-acetotoluidide, 1971, 1972, Toms River;

N-[7-hydroxy-8-[(2-hydroxy-5-nitrophenyl)azo]-1-naphthyl]acetamide, 1971, 1972, Toms River;

7-hydroxy-8-[(4'-[(p-hydroxyphenyl)azo]-3,3'-dimethyl-4-biphenyl)azo]-1,3-naphthalenedisulfonic acid, 1971, 1972, Toms River;

1-(2-hydroxyl-1-naphthylazo)-6-nitro-2-naphthol-4-sulfonic acid, 1971, 1972, Toms River;

1-(2-hydroxy-4-nitrophenylazo)-2-naphthol, 1971, 1972, Toms River;

o-[(p-hydroxyphenyl)-azo]benzoic acid, 1971, 1972, Eastman;

3-[(4-(4-hydroxyphenylazo)-2,5-dimethoxyphenylazo)]-benzenesulfonic acid, 1972, Toms River;

3-hydroxy-4-(phenylazo)-2-naphthoic acid, 1971, Inmont Corp.;

2-(o-nitrophenylazo)-p-cresol (OH = 1), 1972, Toms River;

p-phenylazoaniline (Solvent Yellow 1) and hydrochloride, 1971, 1972, Allied, Am. Cyanamid, duPont;

4-(phenylazo)diphenylamine, 1971, 1972, Eastman;

4-(phenylazo)-1-naphthylamine, 1972, duPont;

5-(phenylazo)salicyclic acid, 1972, Toms River;

N-(p-tolylazo)-sarcosine, 1971, 1972, Blackman-Uhler Chemical Co., GAF;

4-(o-tolylazo)-o-toluidine hydrochloride (Solvent Yellow 3 hydrochloride), 1971, 1972, GAF;

4-(2,4-xylylazo)-o-toluidine, 1971, 1972, Allied;

4-(2,5-xylylazo)-o-toluidine, 1971, 1972, Am. Cyanamid;

4-(2,4-xylylazo)-2,5-xylidine, 1971, 1972, Allied;

4-(xylylazo)xylidines, mixed, 1971, 1972, GAF.

Finally the U.S. Tariff Commission reports that duPont is the only producer of azobisisobutyronitrile (giving no production figures) and that azodicarbonamide is produced by Fairmount Chemical Co., Inc., Stepan Chemical Co., and Uniroyal, Inc. (again giving no production figures for 1971 or 1972, but indicating that 2,060 metric tons were sold in 1971).

III. USE

Azobisisobutyronitrile

Nowak and Rubeus in Kirk & Othmer (1969) stated that this compound - $NCC(CH_3)_2N = NC(CH_3)_2CN$ - was the best known azo compound for free radical initiation of the polymerization of polyesters to resins, but had not yet come into wide commercial use.

Ito (1969) reviewed the properties pertinent to use as a foaming agent for plastics.

La Clair in Modern Plastics Encyclopedia 1972-1973 commented that AIBN, as it is commonly abbreviated, had some use as a chemical blowing agent for polyvinyl chloride foamed plastics, and that it required precaution in handling.

Azodicarbonamide

The Federal Register (1962) announced that this chemical, $H_2NC(O)N = NC(O)NH_2$, had been approved as an aging and bleaching agent in white and whole wheat flours. The limit for such use has since been set at 45 ppm.

Ito (1969) reviewed the properties pertinent to use as a foaming agent for plastics.

La Clair in Modern Plastics Encyclopedia 1972-1973 commented that it was used as a chemical blowing agent for HDPE, PP, PS, and PVC foamed plastics. It was considered to be non-toxic, self extinguishing, and of excellent storage stability.

Azo Dyes

As may be seen from the preceeding section on Production, the usage of azo dyes has been increasing steadily. For many of the major end uses such as natural and synthetic fabrics, paints, plastics, and printing inks, use of any particular dye or color can be highly dependent upon the dictates of fashion, price competition in substrates, packaging material changes, etc. Use in photographic film and enlarging paper is, presumably, more insulated from such "extraneous" concerns. No published breakdown of end use of azo dyes was found.

Usage of azo dyes for foods, drugs, and cosmetics is subject to increasingly intensive toxicological studies; these have resulted in curtailed or discontinued usage for many dyes. These FD&C dyes in a lower purity grade may have uses in general manufacture of dyed goods.

There is no agreement between the United States and Europe in the dyes allowed into and upon the human body. The U.S. is much more restrictive. Bigwood (1973) has analyzed the reports through 1972 of the Joint FAO-WHO Expert Committee on Food Additives. This committee had examined 244 natural and artificial additives with the intent of determining an "acceptable daily intake" in units of mg/kg of body weight. Bigwood came to the conclusion that there had been some hedging in basic definitions, and also raised the important point that it is extremely difficult to determine how much of a particular additive is being ingested except on an individual basis. Only six azo dyes had been studied. A temporary

upper limit of 0.75 mg/kg of body weight had been set for amaranth and Ponceau 4R; Citrus Red No. 2 was not to be used as a food additive; no decision had been reached on Black 7984, Brilliant Black BN, and Orange 1.

Collins and McLaughlin (1972) indicated that about 680 metric tons of amaranth was in annual use in foods, drugs, and cosmetics in over 60 countries.

The newest dye on the FD&C scene seems to be FD&C Red No. 40, which was announced in the 1971 Federal Register. The specifications for food usage once again emphasized the fact that dyes are mixtures and are not discriminated against as such.

Carrière and Luft (1966) examined then current lists of regulated dyes in the U.S.A., W. Germany, France, and Italy, and combined them in tabular form for comparison of all the dyes mentioned in at least one of the country's lists - the French and Italian being non-authorized. These are reproduced here as Table 8.

Table 8. Comparison of U.S. and European FD&C Dye Lists

REDS

Colour Index No.	Federal Designation	Use in U.S.A.	Use in Germany	French No.	Use in France	Use in Italy
16.185	FD & C Red No. 2	A	L–Rot 3	A1 500 E	0–1–2	+
45.430	3	A	C–Rot 38	A1 551 E	0–1	+
14.700	Ext. D & C Red No. 24	A	C–Rot 5	519	0–1–2	
16.150	D & C Red No. 5	B	C–ext. Rot 35	515	1–2	+
15.850	6	B	C–Rot 12	542	1	+
15.850	7	B	C–Rot 12	542	1	+
15.500*	14	B	—	535	1	
26.100	17	B	—	544	1–2–3	
26.125*	18	B	—	533	2–3	+
45.380	21	B	C–Rot 30	547	1	+
45.380	22	B	C–Rot 30	—	—	
45.366	24	B	—	549	1	+
45.410	27	B	C–Rot 34	548	1	+
45.410	28	B	C–Rot 34	548	1	+
45.457*	29	B	—	514	1	+
73.360	30	B	C–WR Rot 12	524	1–3	
15.800	31	B	—	530	1	
15 880	34	B	C–Rot 14	503	1	
12.120*	35	B	C–ext. Rot 1	532	1	+
12 085	36	B	C–Rot 1	520	1	+
12.350*	38	B	C–ext. Rot 4	105	1–2	
13.058	39	B	C–Rot 4	526	1–2	
18 055*	Ext. D & C Red No. 1	C	C–ext. Rot 22	527	2	
16.105*	2	C	C–ext. Rot 19	509	2	+
15.620	8	C	C–ext. Rot 18	523	2	+
14 720*	10	C	—	A1 502 E	2	+
18.050*	11	C	C–ext. Rot 21	528	2	+
27.290	13	C	C–ext. Rot 24	507	2	+
12.141*	14	C	—	539	2–3	
16.155	15	C	—	516	0–1–2	
15.585	D & C Red No. 8	Cx, D, E	C–ext. Rot 17	534	1	+
15.585	9	Cx, D	C–ext. Rot 17	534	1	+
15.630	10	Cx, D	C–ext. Rot 33	536	1	+
15.630	11	Cx, D	C–ext. Rot 33	536	1	+
15.630	12	Cx, D, E	C–ext. Rot 33	536	1	+
15.630	13	Cx, D	C–ext. Rot 33	536	1	+
45.170	19	Cx, D, E	C–ext. Rot 27	521	1–2–3	
17.200	33	Cx, D, E	C–WR Rot 2	512	1–2	
45.170	37	Cx, E	C–ext. Rot 27	545	1	
14.780	—	—	C–Rot 6			
14.830	—	—	C–Rot 7			
15.580	—	—	C–Rot 9			
16.250	—	—	C–Rot 19			
18.000	—	—	C–Rot 21			
18.020	—	—	C–Rot 22	501	1	
18.025	—	—	C–Rot 23			
45.360	—	—	C–Rot 25			
45.380	—	—	C–Rot 30			
45.386	—	—	C–Rot 31			
45.400	—	—	C–Rot 32			
45.405	—	—	C–Rot 33	546	2	+
45.425	—	—	C–Rot 35			
45.435	—	—	C–Rot 36	550	2	+
45.440	—	—	C–Rot 37			
58.000	—	—	C–Rot 41			
16.180	—	—	C ext. Rot 20	506	2	+
27.306	—	—	C–ext. Rot 25			
45.160	—	—	C–ext. Rot 26			
58.005	—	—	C–ext. Rot 31	505	2	
14.895	—	—	C–WR Rot 1			
27.300	—	—	C–WR Rot 5			
68.000	—	—	C–WR Rot 10			
16.050	—	—	—	508	2	
12.315	—	—	—	511	1–2	
45.510	—	—	—	513	2	
16.255	—	—	L–Rot 4	A1 517 E	0–1 2	+
16.290	—	—	L–Rot 5	518 E	1–2	+
—	—	—	—	522	2	
18.810	—	—	—	525	2	
14.830	—	—	—	529	2	
—	—	—	—	531	2	
16.015		—	—	537	2	+
26.105		—	—	538	2–3	
18.065		—		540	2	
—				541	2	
50 240				543	2	
—	—			510	2	

ORANGES AND YELLOWS

Colour Index No.	Federal Designation	Use in U.S.A.	Use in Germany	French No.	Use in France	Use in Italy
45.425	D & C Orange No. 10	B	C-Rot 37	404	1–2	
45.425	11	B	C-Rot 37	406	1–2	
45.456*	14	B	—	413	1	+
45.371*	16	B		401	1	+
11.725*	Ext. D & C Orange No. 1	C	C ext. Gelb 4	410	2–3	+
14.600	3	C		407	2	+
12.100*	4	C		412	2	
15.510	D & C Orange No. 4	D–E	C Orange 2	408	1–2	+
45.370	5	D–E	C-ext. Rot 34	402	1	+
12.075	17	D		411	1–2–3	+
19.140	F, D & C Yellow No. 5	A	L-Gelb 2	Al 217 E	0–1–2–3	
15.985	6	A	L-Orange 2	Al 203 E	0–1–2	
47.005	10	B	L-Gelb 3	208 E	2	
47.000	11	B	C-Gelb 4	Al 215 E	1–2	
13.065	Ext D & C Yellow No 1	C	C-ext. Gelb 10	211	2	
18.820	3	C	C-ext. Gelb 12	209	2	
11.680*	5	C	C-ext. Gelb 2	205	2–3	
14.010*	6	C				
10 316	7	C	C-ext. Gelb 1	212	1–2–3	
45 350	11	Cx	C-ext. Gelb 16	202	1–2–3	
45 350	12	Cx	C-ext. Gelb 16	202	1–2–3	
45.365	—	—	C-Rot 26	403	1–2	+
58.000	—	—	C-Rot 41	400	1–2	
16 230	—	—	C-Orange 5	409	1–2	+
60.515	—	—	C-Gelb 6			
77.199	—	—	C-Gelb 7			
11.920	—	—	C-Orange 1			
15.575	—	—	C-Orange 3			
15.970	—	—	C-Orange 4			
45.395	—	—	C-Orange 7			
71.105	—	—	C-Orange 8			
11.710	—	—	C-ext. Gelb 3			
11.730	—	—	C-ext. Gelb 5			
12.775	—	—	C-ext. Gelb 8			
12.780	—	—	C-ext. Gelb 9			
13 900	—	—	C-ext. Gelb 11			
18.950	—	—	C-ext. Gelb 13	213	2	+
48 055	—	—	C-ext. Gelb 18			
49 005	—	—	C-ext. Gelb 19	218	2	
18.736	—	—	C-ext. Orange 1			
18 745	—	—	C-ext. Orange 2			
18 690	—	—	C-ext. Orange 3			
—	—	—	C-ext. Orange 4			
—	—	—	C-ext. Orange 5			
48.035	—	—	C-ext. Orange 6			
48 040	—	—	C-ext. Orange 7			
29.020	—		C-WR Gelb 1			
29.025	—		C-WR Gelb 2			
65.405	—		C-WR Gelb 3			
65.410	—		C-WR Gelb 4			
68.420	—		C-WR Gelb 6			
40 215	—		C-WR Orange 1			
59.700	—		C-WR Orange 2			
69 025	—		C-WR Orange 3			
69 540	—		C-WR Orange 4			
13.015	—		L-Gelb 1			
14.270	—	—	L-Gelb 4	Al 201 E	1–2	
—	—	—	L-Gelb 6			
75 300	—	—	L-Gelb 7			
41.000	—	—	—	200	1–2	+
25.135	—	—	—	204	2	
11.380	—	—	—	206	2–3	+
11.390	—	—	—	207	2–3	+
10.315	—	—	—	210	2	
19.130	—	—	—	214	2	
—	—	—	—	216	2	
43 395	—	—	—	405	1-2	+
16.020	—	—	—	414	2	

GREENS AND BLUES

Colour Index No.	Federal Designation	Use in U.S.A.	Use in Germany	French No.	Use in France	Use in Italy
42.085	F, D & C Green No. 1	A	—	711	0–1–2	+
42.095	2	A	—	712	0–1–2	+
42.053	3	A	—	710	0–1–2	
61.570	D & C Green No. 5	B	C–Grün 5	701	1–2–3	+
61.565	6	B	C–ext. Grun 4	709	1–2–3	+
42.100*	7	B	C–Grün 2	703	1–2	+
59.040	8	B	C–Gelb 5	700	1–2	
10.020	Ext. D & C Green No 1	C	C–Grun 1	708	2	+
42.090	F, D & C Blue No 1	A	—	7	1–2–3	+
42.090	D & C Blue No 4	B	—	7	1–2–3	+
73.015	F, D & C Blue No. 2	A	L–Blau 2	Al 19 E	0–2	+
73 000	D & C Blue No. 6	B	—			
42.052	7	B	—			
69.825	9	B	—			
52.015*	Ext. D & C Blue No. 1	C	C–ext. Blau 6			
63.010*	4	C	C–ext. Blau 8	2	2	+
42.045	—		C–Blau 2	3	2–3	+
42.735	—		C–Blau 3			
42.750	—		C–Blau 4			
42.755	—		C–Blau 5			
42.135	—		C–Blau 6			
43.820	—		C–Blau 7			
44 040	—		C–Blau 8			
44.045	—		C–Blau 9	14	2–3–4	+
44.075	—		C–Blau 10			
60.730	—		C–Blau 11			
62.085	—		C–Blau 12			
63.000	—		C–Blau 14			
74.180	—		C–Blau 15			
77.007	—		C–Blau 16			
77.510	—		C–Blau 17			
42 170	—		C–Grün 3			
62 550	—		C–Grun 6			
42.080	—		C–ext. Blau 1	17	2–3	
42.140	—		C–ext. Blau 2			
50.315	—		C–ext. Blau 3			
50.320	—		C–ext. Blau 4			
52 015	—		C–ext. Blau 6	12	1–2	+
63.010	—		C–ext. Blau 8			
64.505	—		C–ext. Blau 9			
74.160	—		C–ext. Blau 10	8	2	+
(74.180)	—		C–ext. Blau 11			
10.006	—		C–ext. Grün 1			
42.040	—		C–ext. Grün 2	702	1–2	
42.050	—		C–ext. Grün 3			
74.260	—		C–ext. Grün 5	704	2	+
34.140	—		C–WR Blau 1			
34.230	—		C–WR Blau 2			
62.105	—		C–WR Blau 3			
70.305	—		C–WR Blau 4			
34.270	—		C–WR Grün 1			
77.288	—		C–WR Grün 2			
69.800	—		L–Blau 1			
42.051	—		L–Blau 3			
75 810	—		L–Grün 1			
75.810	—		L–Grun 2			
61.530	—			1	0–2	+
—	—			4	2	
42.770	—			5	2	
43.535	—			6	2	
61.555	—			9	2	+
69.810	—			10	1–2–3	+
51.175	—			11	2	
42 052	—			13	1–2	+
61.525	—			15	2–3	
73.000	—			18	1–2	+
50.405	—			20	2	+
42.000	—			705	2	+
52.020	—			706	2	
44.025	—			707	2	

VIOLETS, BROWNS, BLACKS

Colour Index No.	Federal Designation	Use in U.S.A.	Use in Germany	French No.	Use in France	Use in Italy
42.640	F, D & C Violet No. 1	A	C–Violett 4	804	0–1–2	+
60.725	D & C Violet No. 2	B	C–Violett 7	801	1–2–3	+
60.730	Ext. D & C Violet No. 2	C	C–Blau 11	800	2	+
20.170	D & C Brown No. 1	B	C–Braun 2	104	1–2	+
20.470	D & C Black No. 1	B	C–WR Schwarz 1	302	1–2	+
45.190	—	—	C–Violett 6	803	2	+
45.160	—	—	C–ext. Rot 26	802	1–2	
42.571	—	—	C–Violett 3	805	2	+
16.580	—	—	C–Violett 1	—	—	
43.525	—	—	C–Violett 5	—	—	
61.710	—	—	C–Violett 8	—	—	
73.385	—	—	C–Violett 9	—	—	
61.800	—	—	—	806	2	
42.555	—	—	C–ext. Violett 6	808	2	
42.650	—	—	—	809	2	
42.650	—	—	—	810	2	
42.535	—	—	C–ext. Violett 5	811	2–4	+
—	—	—	—	812	2	
42.745	—	—	C–ext. Violett 7	—	—	
21.010	—	—	—	100	2–4	+
21.010	—	—	—	101	2–4	
20.300	—	—	—	102	4	
21.000	—	—	—	103	4	
12.480	—	—	C–ext. Braun 1	—	—	
13.080	—	—	C–ext. Braun 2	—	—	
20.470	—	—	C–WR Schwarz 1	—	—	
25.040	—	—	C–WR Schwarz 2	—	—	
27.245	—	—	C–WR Schwarz 3	—	—	
35.870	—	—	C–WR Schwarz 4	—	—	
14.805	—	—	C–WR Braun 1	—	—	
28.440	—	—	L–Schwarz 1	—	—	

Notes and Abbreviations

(1) *Use in U.S.A.*

A : F, D & C colours, unrestricted use
B : D & C colours, unrestricted use
C : external D & C colours
Cx : D & C colours, restricted usage, but unrestricted for external D & C usage
D : D & C colours restricted to 6 per cent maximum (pure dye basis) for lipstick use
E : D & C colours restricted to 0·75 mg. maximum ingestion per day, for preparations such as mouthwashes and dentifrices
* Recently delisted by the F.D.A. (*cf.* H. D. Goulden in "Drug and Cosmetic Industry" of February 1965).

(2) *Use in Germany*

L. : food colour, unrestricted use in cosmetics
C : unrestricted use in cosmetics
C–ext. : for external use only : not necessarily safe for ingestion
C–WR : for use in washing and rinsing, or as a solvent or propellant, provided that the material has only transient applicati not necessarily safe when ingested or remaining on the skin.

(3) *Use in France*

0 : completely acceptable for use in any cosmetic including those likely to be ingested (e.g. dentifrices or in mouthwashes)
1 : for external use (lipsticks included)
2 : external colours in the F.D.A. sense of the word
3 : for soaps and non-soapy detergents
4 : for hair preparations
Al : mentioned in "alimentaire décret du 25.3 58"
E : mentioned in "C.E.E. alimentaire décret du 11.11.62"

(4) *Use in Italy*

+ : mentioned by F. Glusotti

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Food Technology (1968) presented a report by Hazelton Laboratories on the use of FD&C colors in food. Tables 9 and 10 here are taken from

this report and indicate the major food categories which use colorants, and amounts of colorants used in them, respectively.

Table 9. Major categories of processed food which use certified FD&C colors in their manufacture, and color concentration levels employed.

Category	Color Concentration, ppm	
	Range	Average
Candy and Confections	10–400	100
Beverages (Liquid & Powdered)	5–200	75
Dessert Powders	5–600	140
Cereals	200–500	350
Maraschino Cherries	100–400	200
Pet Foods	100–400	200
Bakery Goods	10–500	50
Ice Cream and Sherbets	10–200	30
Sausage (Surface)	40–250	125
Snack Foods	25–500	200
Meat Stamping Inks	—	—
Miscellaneous	5–400	—

Table 10. Pounds of primary colors used in foods, drugs and cosmetics. Figures represent sales for the first nine months of 1967 and do not include exports or sales to jobbers and other manufacturers.

	YELLOW No. 5 •	YELLOW No.6 •	RED No. 2 •	RED No. 3	RED No. 4 •	BLUE No. 1	BLUE No. 2	VIOLET No. 1	GREEN No. 3	ORANGE B •	TOTALS
Candy, Confection	59,903	52,770	67,637	11,665	0	6,632	2,499	1,459	124	0	202,689
Beverages	78,933	181,292	282,695	1,056	0	15,800	2,375	985	301	0	563,437
Dessert Powders	59,961	51,622	62,363	8,616	0	3,270	1,659	0	14	0	187,505
Cereals	52,496	35,464	15,558	1,421	0	843	99	0	0	0	105,881
Maraschino Cherries	5,644	4,830	8,104	3,469	11,308	597	0	0	98	0	34,050
Pet Food	101,743	23,226	67,058	1,023	0	1,473	6,764	1,278	0	0	202,565
Bakery Goods	77,885	42,203	43,522	9,560	0	3,680	673	369	7	0	177,899
Ice Cream, Sherbet, Dairy Produce	35,048	23,868	29,697	621	0	2,599	179	45	7	0	92,064
Sausage	6,502	99,605	36,084	4,970	0	647	0	0	0	16,890	164,698
Snack Foods	18,456	11,409	3,623	766	0	305	0	2	0	0	34,561
Meat Inks	15	0	12	10	0	11	0	2,223	0	0	2,271
Miscellaneous	44,841	29,134	46,219	18,200	398	5,345	1,990	1,134	1,298	0	148,559
Subtotal	541,427	555,423	662,572	61,377	11,706	41,202	16,238	7,495	1,849	16,890	1,916,179
Pharmaceutical	17,275	15,938	21,179	12,168	1,186	3,250	593	347	220	0	72,156
Cosmetics	3,125	2,148	3,417	903	630	397	30	96	27	9	10,773
TOTALS	561,827	573,509	687,168	74,448	13,522	44,849	16,861	7,938	3,096	16,890	1,999,108

Note: To convert to metric tons, move decimal three places to the left and multiply by 0.453.

Azo dyes are indicated by a • below the name

The Hazelton report commented that the azo dyes Yellow 5 and 6 and Red 2 comprised about 90% of the total of all food dyes.

Vodoz (1970) in an article on European food additives presented a

Table II.–Food colors permitted (+) in European countries.[1]

COLOR	C.I. 1956 No.	Toxicol. evaluation WHO techn. rep. ser., 309, 1965[2]	E.F.T.A. COUNTRIES							EEC numbers	E.E.C. COUNTRIES						SPECIAL		EASTERN COUNTRIES						
			Austria	Denmark	Norway	Portugal	Sweden	Switzerland	U.K.		Belgium	France	Germany (W)	Italy	Luxembourg	Netherlands	Spain	Turkey	Germany (E)	Bulgaria	Hungary	Poland	Rumania	Czechoslov.	USSR	Yugoslavia
REDS																										
Ponceau MX	• 16150	C_3		+					+[3]																	
Ponceau 4 R	• 16255	C_1	+	+	+		+	+	+	+	124	+	+	+	+	+	+	+	+	+		+		+		+
Carmoisine	• 14720	C_2	+	+	+		+	+	+	+	122	+	+	+	+	+	+	+	+			+		+		+
Amaranth	• 16185	A	+	+	+	+	+	+	+	+	123	+	+	+	+	+	+	+	+	+	+		+	+	+	+
Red 10 B	• 17200	C_2		+					+																	
Erythrosine BS	45430	B	+	+	+		+	+	+	+	127	+	+	+	+	+	+	+		+	+			+		+
Red 2 G	• 18050	C_2		+					+																	
Red 6 B	• 18055	D	+	+					+																	
Red F B	• 14780	D	+						+																	
Ponceau 3 R	• 16155	E				+												+								
Fast Red E	• 16045	C_2	+	+	+		+	+	+	+							+		+					+		
Ponceau 6 R	• 16290	C_2	+	+	+		+			+	126	+	+	+	+	+	+	+	+					+		
Scarlet GN	• 14815	C_2	+	+	+		+	+		+	125	+	+	+	+	+	+	+	+			+				-
Ponceau SX	• 14700	E	+							+								+								
Acid Fuchsine S	—	C_2	+																							
ORANGES and YELLOWS																										
Orange G	• 16230	C_3		+					+																	
Orange RN	• 15970	D		+					+																	
Orange GGN	• 15980	C_2	+	+	+		+			+	111	+	+	+	+	+	+		+				+			
Oil Yellow GG	• 11920	—							+													+				
Tartrazine	• 19140	A	+	+	+	+	+	+	+	+	102	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Naphthol Yellow S	10316	C_3				+																				
Yellow 2 G	• 18965	D		+					+																	
Sunset Yellow FCF	• 15985	A	+	+	+		+	+	+	+	110	+	+	+	+	+	+	+	+			+		+		
Oil Yellow XP	• 12740	D		+					+																	
Acid Yellow	• 13015	C_2	+	+	+		+	+		+	105	+	+	+	+	+	+	+	+		+	+				
Quinoline Yellow	47005	C_2	+	+	+		+	+			104	+	+	+	+	+	+	+	+							
Chrysoin S	• 14270	C_2	+	+							103	+	+	+	+	+	+		+							
GREENS																										
Green S	44090	C_1		+					+		142	+		+	+		+									
Guinea Green B	42085	E				+				+																
Fast Green FCF	42053	B			+		+			+																
BLUES																										
Blue VRS	42045	C_2	+							+																
Indigo Carmine	73015	B	+	+	+	+	+	+	+	+	132	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Indanthrene Bl. RS	69800	C_2	+	+	+		+			+	130	+	+	+	+	+	+	+	+							
Patent Blue V	42051	C_1		+	+		+	+			131	+	+	+	+	+	+									
Brilliant Bl. FCF	42090	B		+														+								
VIOLETS																										
Violet BNP	42580	—		+					+																	
Violet 5BN	42650	C_2								+																
Violet 6 B	42640	C_3			+		+			+																
BROWNS																										
Brown FK	• —	C_2		+					+																	
Chocolate Br. FB	—	D		+					+																	
Chocolate Br. HT	• 20285	C_2		+					+																	
BLACKS																										
Black PN	• 28440	C_2	+	+	+		+	+	+	+	151	+	+	+	+	+	+		+		+	+		+		
Black 7984	• 35445	C_2		+					+		152	+	+	+	+	+	+									

[1] See B.F.M.I.R.A. Information Sheet 281, 1969; and "Food Processing and Packaging Directory 1969–70"

[2] A = Acceptable as a food additive; an acceptable daily intake (or ADI) has been allocated. B = The data are insufficient for justifying "A" classification. C_1 = The data are insufficient for a final evaluation; however, long-term toxicity tests have been done. C_2 = Long-term toxicity not known. C_3 = The data are not sufficient for an evaluation, but there seems to be a risk of nocivity. D = No toxicological data at all. E = The color is not necessary and therefore should not be added to food.

[3] Will be suppressed

table of dyes used in more countries than was given in Table 8 above; his table is reproduced here as Table 11, with the azo dyes denoted by a · to the left of the Colour Index number.

Noonan (1972) reported in Handbook of Food Additives that the then current list of FD&C azo dyes consisted only of Red Nos. 2, 4, and 40, Yellow Nos. 5 and 6, and Orange B. Of these Red No. 4 was restricted for use in maraschino cherries, and Orange B restricted for sausage casings.

Miscellaneous

Shibata (1972) has written an extensive review of the use in the analytical chemistry of polyvalent metal ions of the family of azo compounds derived from the parent 1-(2-pyridylazo)-2-naphthol(PAN).

IV. CURRENT PRACTICE

No information was discovered.

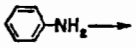
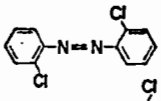
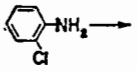
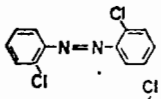
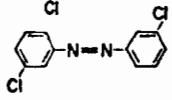
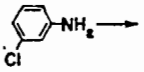
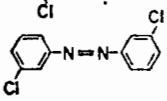
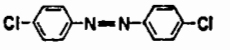
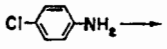
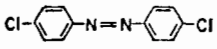
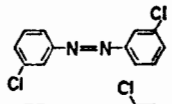
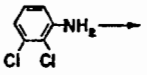
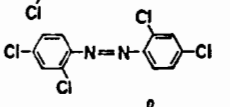
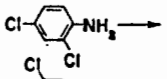
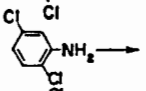
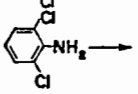
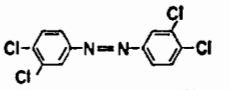
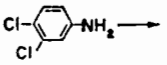
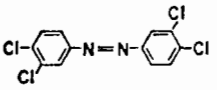
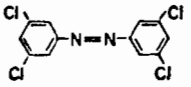
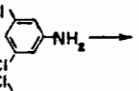
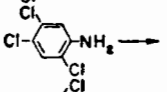
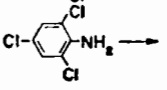
V. ENVIRONMENTAL CONTAMINATION

Bartha and Pramer (1967) were the first to report finding 3,3',4,4'-tetrachloroazobenzene in soil which had been treated with the herbicide 3,4-dichloro-N-propionylaniline (propanil). Since none of the azo compound could be detected in sterilized soil treated with a sterilized solution of the herbicide, the authors concluded that soil microorganisms were at least partially responsible for the transformation. The authors could not find any published reports on the biological activity of the azo compound.

Bartha et al (1968) concluded that peroxidase was the catalyst responsible for the transformation of various chloranilines to chloroazobenzenes in soil. They compared the products resulting from treating chloroanilines in vivo in soil and in vitro in buffered H_2O_2 containing

horseradish peroxidase type II. The results are in Table 12.

Table 12. Formation of Azo Compounds from Anilines in Soil and by Peroxidase

CHLORO-SUBSTITUTION	TRANSFORMATION IN SOIL	ANILINE	TRANSFORMATION BY PEROXIDASE
0	[a]		
2-			
3-			
4-			
2, 3-			
2, 4-			
2, 5-	none [b]		none
2, 6-	none		none
3, 4-			
3, 5-			none
2, 4, 5-	none		none
2, 4, 6-	none		none

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a - Unidentified non-azo aromatic reaction product

b - No reaction occurred

Belasco and Pease (1969) were not able to detect 3,3',4,4'-tetrachloroazobenzene in soil which had been treated for 12 consecutive years with the compound 1,1-dimethyl-3-(3,4-dichlorophenyl)urea (diuron) at 224.6 or 449.2 mg/sq.m. They were able to confirm Bartha and Pramer's initial finding with propanil, at application rates of 250 and 500 ppm (the latter equivalent to 56.15 g/sq.m to a depth of 7.2 cm), in the

laboratory. However, diuron at 500 ppm gave no detectable azo compound. They did not believe that any azo compound formed derived primarily from an aniline precursor.

Bartha (1969) found that soil treated with the herbicides propanil and solan[N-(3-chloro-4-methylphenyl)-2-methylpentanamide] could produce an azo compound derived from portions of both, namely 3,3',4-trichloro-4'-methylazobenzene. This azo compound also resulted from treating soil with 3,4-dichloroaniline and 3-chloro-4-methylaniline. Bartha indicated that because of unequal degradation rates of the two herbicides, the mixed azo compound was unlikely to be produced if propanil treatment preceeded that by solan.

Kearney et al (1970) examined ground used to grow rice and treated with propanil at various recorded times. They found 3,3',4,4'-tetrachloroazobenzene at a level of <0.2 ppm in the top 10 cm of soil treated at the rate of 6.7 kg/hectare.

Sprott and Corke (1971) tested the formation of 3,3',4,4'-tetrachloroazobenzene from 3,4-dichloroaniline in two loamy and two clayey soils under varying conditions of water and oxygen content of the soil. The soil which produced the most azo did so at an optimal temperature of 25°C, an optimal aniline concentration of 500 µg/g, and aerobic atmosphere. In general very little of the aniline which disappeared was converted to the azobenzene, at best only 4.8%. Degradation of the azobenzene in the soils was relatively rapid.

Bordeleau and Bartha (1971) tested the ability of two common soil fungi Penicillium piscarium and Geotrichum candidum to produce tetrachloroazobenzene from propanil. P. piscarium by itself could not metabolize propanil past the dichloroaniline stage. G. candidum by itself could not metabolize propanil at all, but could convert the aniline to

the azobenzene. Together they were able to produce the azobenzene from propanil. The only benefit to the fungi seems to be a reduction in the toxicity of their environment, the azobenzene being less toxic than the propanil or the aniline.

Rosen and Siewierski (1971) prepared a derivative of 3,3',4,4'-tetrachloroazobenzene in which one of the P-chloros has been replaced by the nitrogen of 3,4-dichloroaniline. This derivative had earlier been proposed as a degradative product of propanil. The authors demonstrated that the derivative was stable after at least two months in soil capable of converting the aniline to the tetrachloroazobenzene; the derivative in methanol was resistant to two weeks of exposure to glass-filtered sunlight, and to ten hours of exposure to UV light of wavelength over 297 nm.

Helling (1971) found that the Rf values in a soil sample were 0.24 for propanil, 0.22 for 3,4-dichloroaniline, and 0.00 for 3,3',4,4'-tetrachloroazobenzene.

Briggs and Ogilvie (1971) reported that 3-chloro-4-methoxyaniline was converted in soil into 3,3'-dichloro-4,4'-dimethoxyazobenzene by a free radical mechanism.

Child et al (1972) reported that 3,3',4,4'-tetrachloroazobenzene was optimally effective at the 81 mg/kg level against adenocarcinoma tumors in mice, nearly tripling the survival time of animals receiving mammary tumor transplants.

Burge and Gross (1972) found that 3,3'-dichloro- and 3,3',4,4'-tetrachloroazobenzene were satisfactorily extracted from a variety of soil samples with 95% ethanol. Analysis by gas-liquid chromatography using a micro-coulometric detector did not require evaporation of the alcoholic extract.

Kearney and Plimmer (1972) studied the effect of 3,4-dichloroaniline concentration in soil on tetrachloroazobenzene generation. Between 1 and 100 ppm there was about a twofold increase in azobenzene for each tenfold increase in aniline; between 100 and 1000 ppm there was only a 10% increase in azobenzene. They also seemed to have isolated both cis and trans forms of the azobenzene by thin layer chromatography.

Bordeleau et al (1972) studied the conversion of 3,4-dichloroaniline to 3,3',4,4'-tetrachloroazobenzene in a H_2O_2-peroxidase system with the intent of determining the intermediates. Their results are represented in Figure 1. The main pathway involved intermediate (II), but there was evidence for the presence of the free radical (III).

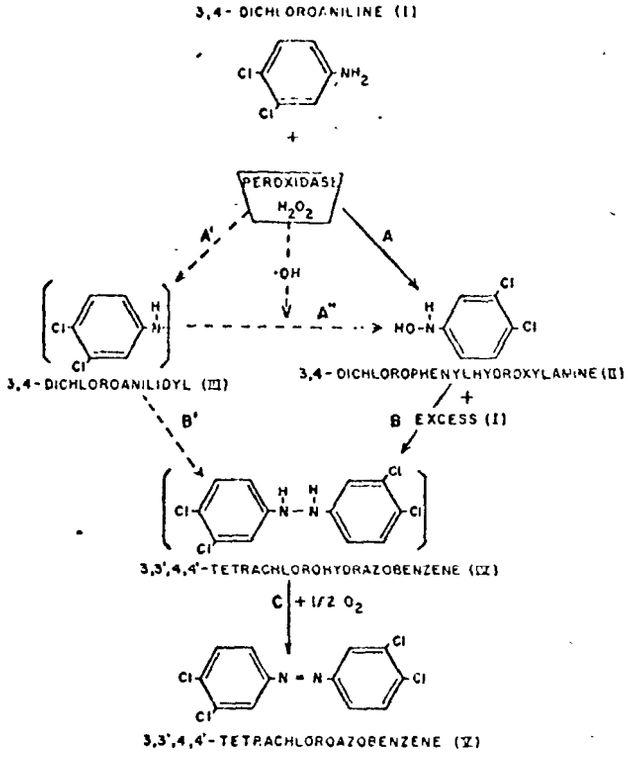


Figure 1. Proposed pathway of 3,3',4,4'-tetrachloroazobenzene formation.

VI. MONITORING AND ANALYSIS

A. Analysis

Spain and Clayton (1955) analyzed for dyes in feces, tissue, and urine by alkaline digestion, acidification, filtration, colorimetric measurement, reduction with stannous chloride, and remeasurement of color.

Parsons and Seaman (1955) described a method of external generation at a mercury pool electrode of the Ti(III) used to titrate azo dyes. Comparison of this coulometric titration with the standard titration with Ti(III) using technical grades of Orange II, tartrazine, and p-aminoazobenzene was favorable..

Feigl and Neto (1956) described a spot test for azo dyes whose structures incorporate a p-phenylenediamine or a p-nitroaniline moiety. Fusion of the dye with a mixture of sodium formate/sodium hydroxide at about 220°C causes the release of p-phenylenediamine by sublimation (from both structural types). The diamine vapor reacts with aniline in an oxidizer solution to form a color. Minimum quantities of sample required are 5-10 μg.

Mecke, Jr. and Schmahl (1957) reported ultraviolet absorption maxima for over two dozen aromatic azo compounds.

Earley and Ma (1960) expounded upon the Ti(III) titration of azo compounds, its applicability in the presence of nitro compounds, and alterations in the standard technique required.

Sawicki et al (1961) developed a spot test for aminoazobenzene and its N-methyl and ethyl derivatives which involved reaction with the compound 3-methyl-2-benzothiazolone hydrazone (MBTH). Table 13

Table 13. Spectrophotometric Determination of 4-Aminoazobenzene Derivatives and Azobenzene Analogs[a]

Compound	λ_{max}, mμ	ε × 10^{-3}
4-Aminoazobenzene Derivatives		
4-Aminoazobenzene = AB	571	11
	611s	5
N-Methyl AB	580	36
	630	31
	664s	32
3′-Methyl-*N*-methyl AB	586	36
	625s	34
	664s	32
N-Ethyl AB	605	67
	666s	53
N-Phenyl AB	623	39
4′-Methylthio-*N*-phenyl AB	624	9
N,*N*-Dimethylaminoazobenzene = DAB[b]	604	70
	672	56
2-Methyl DAB	578	72
	664s	20
2′-Amino DAB	608	45
	660s	37
2′-Chloro DAB	613	73
	664s	61
2′-Ethyl DAB	603	67
	665s	47
2′-Methoxy DAB	605	64
	665s	51
2′-Methyl DAB	603	69
	671s	45
2-Methyl-2′-methoxycarbonyl DAB	585s	28
	665s	8
2,3′-Dimethyl DAB	588	71
	670s	11
2-Methyl-3′-chloro DAB	588	71
	665s	19
2-Methyl-4′-acetyl DAB	557	65
	666s	13
2-Methyl-4′-methylthio DAB	585	74
	670s	10
2′,5′-Dimethyl DAB	601	66
	665s	43
2′,4′,6′-Tribromo DAB	612	5
	674s	4
N-Methyl-*N*-ethylaminoazobenzene = MEAB	603	58
	663s	32
2′-Chloro MEAB	605	63
	659s	51
2′-Nitro MEAB	613	42
	665s	31
3′-Acetamino MEAB	603	59
	659s	33
3′-Nitro MEAB	605	54
	660s	33
4′-Ethyl MEAB	600	60
	665s	29
4′-Fluoro MEAB	601	61
	663s	33
N-Methyl-*N*-benzyl AB	607	10
	675s	4
N,*N*-Diethyl AB	570s	32
	600	38
	671s	5
3′-Acetamino DAB	605	70
	664s	55
3′-Amino DAB	588	50
	672s	25
3′-Chloro DAB	608	66
	667s	51
3′-Ethoxy DAB	603	70
	663s	51
3′-Methyl DAB	603	68
	664s	52
3′-Nitro DAB	610	51
	665s	43
3′-Trifluoromethyl DAB	608	69
	663s	57
4′-Acetyl DAB	606	25
	666s	20
4′-Amino DAB	585	41
	668s	11
4′-Ethoxy DAB	598	49
	665s	29
4′-Ethyl DAB	603	59
	662s	43
4′-Fluoro DAB	603	37
	664s	26
4′-Methyl DAB	601	45
	666s	31
4′-Sulfo DAB	612	57
	666s	40
4′-Thiocyano DAB	599	18
	668s	12
2,2′-Dimethyl DAB	588	71
	665s	15
Azobenzene Analogs		
4-Phenylazo-1-naphthylamine	587	40
1-[*N*,*N*-Dimethyl-4-aminophenylazo]-naphthalene	608	66
	662s	55
2-[*N*,*N*-Dimethyl-4-aminophenylazo]-fluorene	573	12
	680s	4

[a] All values based on minimum of two determinations and are within ±2% of average. [b] Molar absorptivity values based on 36 determinations giving a value of 70,000 ± 1000 at λ_{max} 604 mμ.

gives the compounds which react with MBTH. These compounds did not react completely: 4'-acetyl-N,N-dimethylaminoazobenzene (4'-acetyl-DAB), 4'-methylmercapto-N-phenylaminoazobenzene, 2',4',6'-tribromo-DAB, 4'-thiocyano-DAB, and 2'-methoxycarbonyl-2-methyl-DAB. These compounds did not react al all: azobenzene, 4-hydroxyazobenzene, 4-methylmercaptoazobenzene, and 4'-nitro-N,N-diethylaminoazobenzene. These compounds gave a reaction, but the product had a molar absorptivity less than 4,000: 4'-nitro-N-phenylaminoazobenzene, 4'-phenyl-DAB, 4'-nitro-N-methyl-N-ethylaminoazobenzene, 3-methyl-DAB and N,N-dimethyl-p-(p-2-tolylazo-2-tolylazo)aniline.

Villanúa et al (1962) presented in tabular and graphic form the UV spectra in acidic, alcoholic, and alkaline solution of the banned-for-food water insoluble azo dyes: Methyl Red, Sudan I, II, III, and IV, Orange SS, Yellow AB and OB, o-aminoazotoluene, and 4-dimethylaminoazobenzene. Rf values for circular paper chromatography were also given.

Chikryzova and Podolenko (1964) presented a method suitable for monitoring the concentration of a known dye. It involved reduction at a Hg cathode, and titration of iodine liberated at the anode. Interference from oxygen traces did not become bothersome until the dye concentration dropped below 1 μM.

Bowie et al (1967) presented in tabular form the mass spectra of a wide variety of substituted azobenzenes.

Venturini (1967) detected acidic azo dyes in wine by adsorption on polyvinylpyrrolidinone.

Gemzova and Gasparic (1967) analyzed disperse azo dyes by reductive cleavage of the azo linkage with Zn in hot acetic acid, followed by

paper or thin-layer chromatography of the released amines. Rf values for many amines were given, along with those for DNP-hydrazones of diamines or amino-phenols which were first oxidized to quinones.

Oi and Inaba (1967) used infrared spectroscopy to identify amaranth, New Coccine, Orange 1, Ponceau SX, and Sunset Yellow. In order to use NaCl cells, aqueous solutions of the dyes were extracted with 5% Amberlite LA-2 (a high molecular weight amine) in CS_2. Characteristic absorption bands were given.

Manukian and Mangini (1971), by various spectroscopic and direct synthetic means, identified the dye Colour Index Pigment Red 178 as the reaction product of two molecules of p-aminoazobenzene with perylene-tetracarboxylic acid dianhydride, the amine N's having displaced the ether O's of the anhydrides.

B. Separation-Analysis

Edwards, Jr. et al (1956) reported R values on various adsorbents using one or more eluants for p-hydroxyazobenzene*, p,p'-azophenol*, p,p'-azodimethylaniline*, N,N-dimethyl-p-phenylazoaniline, azobenzene, p-chloroazobenzene, p-methylazobenzene, and p-phenylazobenzoic acid (melting points given for compounds with asterisk).

Fukui et al (1956) studied the paper chromatographic separation of p-amino-, p-methylamino, and p-dimethylaminoazobenzene using water mixed with a variety of alcohols, ethers, acetone, methylnitrile, and amines; Rf values and judgments as to suitability of the various eluants were given.

Ward et al (1959) chromatographed a variety of azo compounds prepared from diazotized p-nitroaniline or beta-naphthylamine with alpha-

naphthylamine, beta-naphthylamine, 5- and 8-nitro-alpha-naphthylamine. Ultraviolet absorption maxima, minima, and log ε's, along with some melting points were tabulated.

Fore and Walker (1967) used a combination of paper and thin layer chromatography, and synthesis to determine the composition of the (foreign) food dye Brown FK. The two major components were 2,4-diamino-5-(p-sulfophenylazo)toluene(I) and 1,3-diamino-4,6-bis-(p-sulfophenylazo)-benzene(III). In lesser quantity was 1,3-diamino-4-(p-sulfophenylazo)-benzene(II). In still lower amounts were 2,4-diamino-3,5-bis(p-sulfophenylazo)toluene(IV) and 1,3-diamino-2,4,6-tris(p-sulfophenylazo)-benzene(VI). A trace of 1,3-diamino-2,4-bis(p-sulfophenylazo)benzene(V) was found. In addition two unidentified, colorless components were found. The two major components did not result from the dye synthesis as accidental byproducts, but were intentionally formed. Some evidence was presented for the necessity of some of the minor components in the successful use of the dye. Figure 2 presents the structures of the identified components.

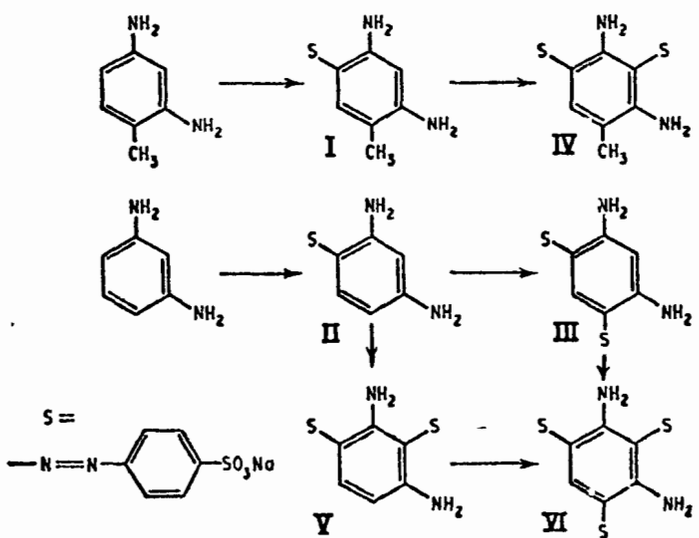


FIG. 2. Structures and synthetic pathways of Brown FK components.

Brain et al (1971) chromatographed amaranth and Sunset Yellow FCF following British Standards. Depending upon the manufacturer the main peak in amaranth represented 78.4-99.7% of the whole, the impurities, including the azo dye Fast Red E, also showing variations. In the case of FCF the major component comprised 93.6-98.6% of the whole, but the impurities showed a greater range. The impurities seemed to be characteristic of a particular manufacturer and could be used to identify him.

Marmion (1972) described an AOAC method of analyzing FD&C Yellow No. 6 for traces of the materials from which it is synthesized: sulfanilic acid (diazotized) and 6-hydroxy-2-naphthalenesulfonic acid (Schaeffer's salt), and for an impurity in the latter, 6,6'-oxybis(2-naphthalenesulfonic acid). The method involved column chromatography over cellulose using 20 and 40% aqueous diammonium sulfate as eluant, followed by UV analysis of the eluates. The impurities eluted in the order in which they were mentioned above.

C. Separation

Birnbaum et al (1953) separated the cis and trans isomers of azobenzene and derivatives by irradiating a petroleum ether solution with a Hg arc and filtering over an alumina column which retained only the cis. They obtained UV spectra for the trans and stable cis isomers. Melting points were given for the isomer pair of the para derivatives: iodo, bromo, chloro, fluoro, ethoxy, methyl, carborethoxy, nitro, carboxy, cyano, dimethylamino, and p,p'-dimethyl.

Frankel and Wolovsky (1954) separated the cis and trans isomers of azobenzene by paper chromatography using the eluant 40% acetic acid.

Silk (1963) presented a column chromatographic method, on Celite, for separating lipstick dyes.

Topham and Westrop (1964) found that thin layer chromatography on silica gel G, developed with 95/5 chloroform/methanol, provided an adequate separation of: 4-aminoazobenzene (AB), 4'-hydroxy AB, N-methyl AB, 4'-hydroxy-N-methyl AB, N,N-dimethyl AB, and N,N-dimethyl-4'-hydroxy AB. An appropriate range for detection in a mixture was 25 ng-1 µg.

Gurevich and Chukreeva (1967) presented Rf values on four activity grades of silicic acid for azobenzene, p-aminoazobenzene, p-hydroxyazobenzene, p-methoxyazobenzene, Sudan yellow, and Sudan red. Any of the grades seemed suitable for the separation of a mixture of all six using carbon tetrachloride.

Parrish (1968) found that Sephadex G-25 was suitable for the separation of azo dyes. Judicious adjustment of the ionic strength of the water used as eluant provided a means to alter Rf values, and thus separate complex mixtures using more than one column or pass.

Naimy et al (1969) reported Rf values on silica gel for the cis and trans forms of 4-amino-4'-ethylazobenzene with 0, 1, or 2 methyl groups on the amine, and 2 or 4 fluoro atoms on the amino phenyl ring. Considering the precautions taken to isolate these geometrical isomers, it appears that most previous publications reporting Rf values dealt with mixtures.

Hall and Perkins (1971) disclosed a procedure for purifying commercial dyes of isomers and color standardization adjuvants. Essentially it consisted of extracting either an aqueous solution of the dye with acidified butanol, or the undissolved dye with hot N,N-dimethylformamide in

cases of butanol insoluble dyes. Impurities or additives remained behind or were selectively retained in the extractant while the dye was re-precipitated.

Gasparic (1972) discussed paper and thin layer chromatography of azo pigments and lakes. Warm N,N-dimethylformamide was the best solvent for spotting these sparingly soluble substances, but it had to be thoroughly removed before proceeding with development by benzene or toluene. More polar developers were required for the lakes.

Gilhooley et al (1972) described procedures for extracting food dyes from water soluble foods, baked goods, and processed meats. The extracts were then chromatographed over polyamide columns to further purify the dyes, prior to identification by known thin layer chromatographic techniques.

VII. CHEMICAL REACTIVITY

A. Environmental and use associated reactions

In the application of dyes to whatever is intended to be colored, chemical reactions are sometimes employed but they do not involve the azo linkage. The amphoteric protein nature of wool and silk renders them easily colored by dyes having amine or sulfonic acid groups through salt formation. Dyes containing these groups and/or hydroxy or carboxy groups can be fixed to cotton by a process called mordanting. This involves first reacting the cotton with a metal oxide (for acid groups) or tannic acid/tartar emetic (for basic groups). Again, simple acid-base reactions are involved. Generation of the azo group, and thusly the dye, on cotton itself has been practiced nearly a hundred years. Ir "ice coloring" a phenol is soaked into the cotton, and then reacted with an iced diazonium solution. In "ingrain dyeing" an amine is first applied to the cotton, then diazotized, and finally immersed in a phenol solution. A dye type for cellulosics that covalently binds to the fiber has been developed in the last 15-20 years, and consists usually of a suitable dye which has been modified by the addition of a dichlorotriazinyl group. The dyeing takes place in an alkaline medium to assist the displacement of one of the chlorine atoms by one of the hydroxyl oxygen atoms of the fiber. Alternatives to the dichlorotriazinyl group are vinylsulfonyl ($\cdot SO_2-CH = CH_2$) and activated alkyl hydrogen sulfate; both give ether linkages with the fiber

in the same fashion as the triazinyl.

When used as chemical foaming agents the two compounds azodicarbonamide, $H_2NC(O)N = NC(O)NH_2$, and azobisisobutyronitrile (AIBN), $[NCC(CH_3)_2N=]_2$, decompose when heated to give off nitrogen gas. The residual parts of the molecules may decompose further or combine with one another. When the AIBN is used to initiate polymerization, it breaks apart into $NCC(CH_3)_2N\cdot$ radicals. These eventually recombine, combine with H· radicals, or other radicals generated in the overall process or present on equipment walls.

Mytelka and Manganelli (1968) demonstrated that irradiation with a Co-60 source of the mother liquor from production of Direct Red 79, assisted by oxygenation, decolorized the liquid and reduced its oxygen demand. Furthermore, the treated waste became more biodegradable.

Trimmer (1971) reviewed the recent literature and also reported the results of his own studies on purifying textile plant wastes of dyes. A variety of oxidative methods, among some non-chemical ones, were tried but it did not look as if any one treatment method could be considered generally applicable.

Evans (1971) indicated that azobenzene, in an acidic, dilute ethanol solution, was converted by sunlight via the Beckmann rearrangement into benzidine (probably far more toxic than azobenzene) and via intramolecular ring closure into benzo[c]cinnoline (9,10-diazaphenanthrene).

Van Beek et al (1971) in a study of 18 azo dyes in water or ethanol solution found that flash photolysis in the presence of a proton donor first produced -NH-N· radicals. Two of these disproportionated to form -NH-NH- and -N=N-. The -NH-NH- decomposed to $-NH_2$ or reverted to -N=N-.

B. Aspects with biological implications

Cilento (1952) found that o-aminoazotoluene, p-diethylaminoazobenzene,

and 3'-methyl-dimethylaminoazobenzene formed complexes with bile acids.

von Euler et al (1952) heated p-dimethylaminoazobenzene with cysteine·HCl in methanol. They recovered 1/4 of the dye unchanged, and 1/4 as 1,4-diaminobenzene. In another experiment incubation of the dye with ground rat liver for two hours at 37°C resulted in complete loss of color.

Kawai (1952) studied the decomposition of p-aminoazobenzene, p-methylaminoazobenzene, and o-aminoazotoluene by fresh slices of rat liver or kidney. The N-methylated dye decomposed at a slower rate than the other two. The liver showed the greater capacity for decomposition. Supplementation of the basic rice diet with yeast, liver powder, or liver extract improved the ability of liver slices to decompose the dyes. Spiking of the slices with riboflavin likewise increased this.

Diemair and Boekhoff (1953) studied the inhibition of pepsin (in gastric juice) by azo dyes. The order of decreasing inhibition found was: Orange GG, Brilliant Black = Naphthol Red S, Ponceau 6 R, Fast Yellow Extra, Bordeaux R = Orange SXX = Tartrazine XX, Cochineal Red A = Yellow 27175, Fast Red E, Thiazine Brown R. The concentration range 50-1000 mg/l was used, the Orange GG showing 3% inhibition at the low end, and the Thiazine Brown R only 13% at the high end. Total inhibition at the high end was found with the first four dyes mentioned. In a follow up study on inhibition of trypsin, the dyes Bordeaux R, Brillian Black, and Orange SXX were found effective.

Burkhard et al (1953) found that the binding of albumin from bovine serum to 4-amino-4'-sulfoazobenzene was slight, but increased if the amino group was removed or alkylated.

von Euler et al (1954) reported on the metabolism of p-dimethyl-

aminoazobenzene by rats. When incubated for 24 hours with 1 g of normal rat liver, as much as 40 µg of dye was totally used up. When cancerous liver was used under the same conditions, 0, 55, and 75% of 10, 20, and 40 µg quantities of dye, respectively, remained. Homogenized intestine in 24 hours used up 99-100% of the dye. There appeared to be some limit on the ability of the rat to clear the dye from its stomach as 2.3 mg remained 12 hours after introduction of 12 mg; for comparison, 0 mg remained 23 hours after dosing with 18 mg. Blood concentration of azo dyes, 6.4 µg/g, was found 20 hours following an oral dose of 12 mg of the dye at the conclusion of a 55 day period during which 540 mg of dye was administered, orally. The liver contained 0.35 µg/g of a mixture of the dye and its mono- and di-demethylated metabolites, at the end of an eight month period of daily oral dosing with 6 mg dye.

Hatem (1959) reported that histamine formed complexes with the carcinogens: p-aminoazobenzene, 2',3-dimethyl-4-aminoazobenzene, and 3'-methyl-4-dimethylaminoazobenzene.

Kusama (1960) introduced into the stomachs of male rats the carcinogenic azo compounds: p-N,N-dimethylaminoazobenzene, 2',3-dimethyl-4-aminoazobenzene, p-aminoazobenzene, p-N-methylaminoazobenzene, and 3'-methyl-4-N,N-dimethylaminoazobenzene. He then showed that the dyes were bound to tyrosine molecules in the liver.

Matsumoto and Terayama (1961) compared the rates of N-demethylation by rat liver homogenate of various N- and ring-methyl-substituted p-methyl, alkylaminoazobenzenes. They did not find any correlation between the rates and the known carcinogenicities.

Matsumoto (1961) used homogenized rat liver to N-demethylate a variety of C- and N-alkylated p-aminoazobenzenes. It was found that those

compounds which resisted demethylation were also non-carcinogens; however, only some of those which did demethylate were carcinogens.

Ishidate and Hanaki (1961) conducted non-enzymic oxidative N-demethylations of ring methylated p-N,N-dimethylaminoazobenzene. They found that the reaction rates correlated with the pi-electron density at the amino N, but not with carcinogenicity.

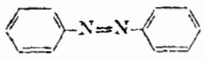
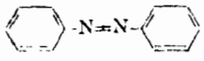
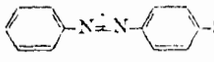
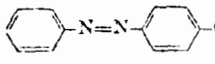
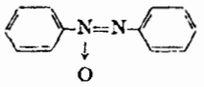
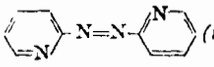
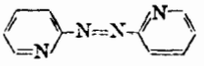
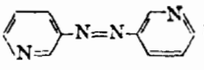
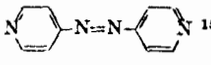
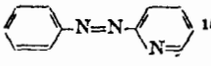
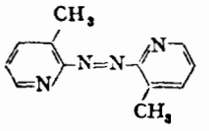
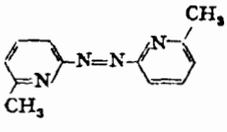
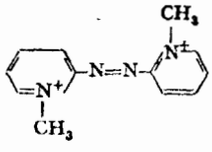
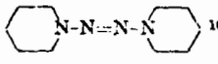
Callander and Roberts (1961) studied the ability of hydrogenase from Azotobacter vinelandii and Desulfovibrio desulfuricans to catalyze the reduction of azo linkages. The compounds tested and the results are given in Table 14. A + means that reduction occurred. A + in the Indirect column means that a "carrier", benzyl viologen (from 0.5-1 part per 1000 parts of azo compound), was required for reduction to occur. Seemingly, direct reduction was related to the ability of the groups bonded to the azo linkage to withdraw electrons from it.

Sikorska and Krauze (1962) studied the effects of FD&C dyes on the activity of succinic oxidase from rat liver (homogenized). At the level of 4 μg dye/mg rat liver only food Black No. 1 showed any effect, an 8-16% inhibition. At 400 μg/mg: chrysoidine had no effect; food Yellow Nos. 3 and 4 had an inhibition of <20%; direct Blue No. 5, food Red Nos. 2, 3, and 7 had an inhibition of 20-50%.

Manchon et al (1962) studied the effect of methyl orange on oxygen consumption of various substrates and rat liver homogenate or a supernatant thereof. Using homogenate and glucose-6-phosphate (3.3 mM) there was a 17% decrease when the concentration of dye was 14-86 μM; using supernatant the decrease was 40%. On a different strain of rats these figures were 11 and 32%, respectively. Using homogenate, 10 mM α-ketoglutarate, citrate, or succinate, and 0→195 μM dye, there was a 36% decrease for the ketoglu-

Table 14. Reduction of Azo Bonds by Hydrogenase

THE CONCENTRATION OF SUBSTRATE WAS 10^{-2} *M* IN ALL CASES

Substrate	Reduction: Direct	Reduction: Indirect	Remarks
$CH_3-N=N-CH_3$[13]	—	—	
$(CH_3)_2CH-N=N-CH(CH_3)_2$[14]	—	—	
 *(trans)**	—	+	
 *(cis)*[15]	—	+	
 *	—	+	70 % inhibition of enzyme at a concentration of 10^{-2} *M*
 *	—	+	
 *	—	+	
 *(trans)*[11,12]	+	+	Stimulated by Fe^{2+}, Cu^{2+}, Co^{2+}, Hg^{2+}, Cr^{3+}
 *(cis)*[15]	+	+	Slow rate of reduction (10 % of rate of reduction of *trans*)
 [15]	+	+	Rate of reduction same fo 2,2′-isomer. No metal stimulation
 [15]	+	+	
 [15]	+	+	
	+	+	Low rate of reduction: approx. 5 % of rate of reduction of 2,2′-azopyridine
	+	+	
	+		Rapid reduction: approx. 20 × that of 2,2′-azopyridine
 [16]	—	—	

tarate (mostly reached at about 60 μM dye), nominal decrease for the citrate, and a 53% increase for the succinate (still rapidly increasing at 195 μM). With the substrate D-phenylalanine (20 mM) and 86 μM dye, a noticeable decrease in oxygen consumption occurred.

Manchon and Lowy (1962, pp. 1619-22) demonstrated that the ability of rat liver homogenate supernatant to reduce the azo linkage of methyl orange was increased when the rat had been allowed to drink water containing 0.2 g/l of the dye for 18-39 days. However, this higher activity supernatant was inactive against ethyl orange.

Matsumoto and Terayama (1965) compared the rates of reduction by rat liver hydrogenate of the azo linkage of ring-methylated mono- and dimethylaminoazobenzenes, and also p-R_2N-azobenzene (where R_2 is all possible combinations of H, Me, and Et). They found nc correlation between the reduction rate and carcinogenicity, or pi-electron density at the azo linkage.

Matsumoto and Terayama (1965, pp. 331-7) gave rats oral doses of various derivatives of p-aminoazobenzene. From the livers they extracted dyes indicative of N-dimethyl types breaking down to N-methyl and $-NH_2$, and of N-methyl types breaking down to $-NH_2$ or disproportionating to $-NH_2$ and N-dimethyl. Those dyes having $-NH_2$ to start and methyl groups on one of the phenyl rings were not able to produce N-methyls or N-dimethyls.

Matsumoto and Terayama (1965, pp. 339-51) found that one of the liver metabolites from feeding 2',3-dimethyl-4-aminoazobenzene to rats was 4,4'-bis(o-tolylazo)-2,2'-dimethylazobenzene, apparently resultant from oxidative coupling of the 4-amino group of the parent (also known as o-aminoazotoluene).

Higashinakagawa et al (1966) studied the degradation in rat liver of orally administered N,N-dimethylaminoazobenzene. Apparently the first step was loss of one of the methyls. Then the remaining methyl became coupled to the sulfur atom of a methionine residue, thereby generating the precursor to the polar dye commonly extracted from the rat liver after chemical decoupling of the methionine from its protein tail.

Westrop and Topham (1966) found as liver metabolites in rats fed 4-dimethylaminoazobenzene: 4-methylaminoazobenzene, 4-aminoazobenzene, 4'-hydroxy-4-dimethylaminoazobenzene, 4'-hydroxy-4-methylaminoazobenzene, 4'-hydroxy-4-aminoazobenzene, and 4'-hydroxy-4-acetylaminoazobenzene. The same four hydroxylated metabolites were found when 4'-fluoro-4-dimethylaminoazobenzene was used, but in lower amounts. This ready displacement of the fluoro atom was unexpected, and it raised some concern about the validity of studies which had introduced fluorine atoms at various positions on the phenyl rings of azobenzenes to determine if these positions were actively involved in carcinogenesis.

Westrop and Topham (1966, pp. 1395-9) found that there was no correlation between carcinogenicity and total 4'-hydroxylated metabolites in the liver of rats fed 3-methyl-4-methylaminoazobenzene,4-dimethylaminoazobenzene, and the latter's 2-,2'-, or 3'-methyl derivatives. In contrast there was a correlation with these 4'-substituted derivatives: chloro, ethyl, fluoro, methoxy, methyl, nitro, and trifluoromethyl. The authors hypothesized that the hydroxylation occurred initially on the amine N after one demethylation, followed by an internal rearrangement of the hydroxy to the 4' position. It had not been possible to prepare the intermediate N-hydroxy to test this.

Turba et al (1966) incubated homogenized rat liver with 3'-methyl-

N,N-dimethyl-4-aminoazobenzene which had a C-14/H-3 ratio of 0.60. This ratio was 0.43 and 0.62 in the cytoplasmic and microsomal protein fractions, respectively, of the post-incubation homogenate. This difference was interpreted as indicative of N-demethylation occurring while the dye was bound to the cytoplasmic protein.

Daniel (1967) examined the comparative rates of azo linkage reduction of tartrazine, Orange II, and Orange G by rat liver homogenate supernatant, and found them to be 1, 2.5, and 3.4, respectively (male rats), or 1, 2.5, and 2.9, respectively (female rats). The difference between male and female rats in the reduction of Orange G was deemed of low significance because of wide variance.

Lolua et al (1967) commented that iron was capable of reducing azo linkages in acid media to the hydrazo (-NH-NH-) or amine stage. They found that 0.1% of sorbic acid was sufficient to protect amaranth, chrysoidine, and tropaeolin.

Ryan et al (1968) tested the ability of rat liver homogenate and protein preparations from the intestinal bacteria E. coli and Proteus vulgaris to reduce the azo linkage of a variety of water-soluble azo dyes. Their results are presented in Table 15, and are indicative of metabolism of common food and drug dyes occurring in the intestine, not the liver.

Table 15. Reduction of water-soluble azo dyes by rat liver homogenate supernatant and soluble bacterial preparations

Dye	Color Index No.	Percentage reduction*		
		Liver	Bacterial	
			Proteus	E. coli
Tartrazine	19140	4.0	54	24
Lissamine fast yellow 2G	18965	17.0	--	--
Amaranth	16185	76	95	91
Ponceau SX	14700	0	85	81
Fast yellow	13015	41	95	91
Naphthalene fast orange 2G	16230	9.0	95	92
Sunset yellow	15985	11.0	95	95
m-Methyl orange	--	72	93	60
Neoprontosil	--	--	95	60

* Incubated anaerobically, and assayed after 60 min. Corrected for protein binding.

The incubation medium contained liver homogenate equivalent to 250 mg wet weight, or soluble bacterial protein (2 mg) together with dye (1 μM), $MgCl_2$ (2 μM), NADP (300 μM), glucose-6-phosphate (250 μM), glucose-6-phosphate dehydrogenase (1 Kornberg unit) in 0.07 M phosphate buffer, pH 7.4.

Maher et al (1968) allowed N-benzoyloxy-N-methyl-4-aminoazobenzene and biologically active DNA from Bacillus subtilis tc interact at room temperature and pH 7.5. As a result the DNA suffered severe reduction in transforming activity, increase in frequency of mutation, and decrease in buoyant density. None of these changes resulted from contact of the DNA with 4-methylaminoazobenzene.

Lin et al (1968) identified the polar dye P2b from the liver of rats fed 4-methylaminoazobenzene as 3-(homocystein-S-yl)-4-methylamino-azobenzene.

Wu and Smuckler (1968) isolated rat liver microsomes and incubated them with 4-amino-, 4-methylamino-, and 4-dimethylaminoazobenzene. Cleavage of the azobond was much faster in the monomethylated compound. Demethylation rate was equivalent. The optimal cleavage conditions did

not produce any demethylation. Rate of cleavage was always greater than rate of demethylation, apparently just the opposite of the situation in intact animals.

Lin et al (1969) reacted tyrosine with N-benzoyloxy-N-methyl-4-aminoazobenzene to give a pair of polar dyes. These were shown to be identical (spectroscopically, chromatographically, and chemically) with the two polar dyes, commonly designated P1a and P1b, which may be isolated from the liver of rats fed N-methyl-4-aminoazobenzene. P1a was temporarily described as N-(3-tyrosyl)-N-methyl-4-aminoazobenzene, and P1b as 3-(3-tyrosyl)-N-methyl-4-aminoazobenzene. By oxidation with hydrogen peroxide the authors were able to convert synthetic 3-(homocystein-S-yl)-N-methyl-4-aminoazobenzene into a sulfoxide which was identical with the minor polar dye P1c. These four dyes P1a, P1b, P1c, and P2b comprise 90% of the polar dyes derived from hepatic protein-bound dyes by successive enzymic and hot alkaline hydrolysis.

Matsumoto and Terayama (1970) gave rats p-methyl-, and dimethyl-, and methyl ethyl aminoazobenzenes in which the carbons and/or the protons on the carbons of the alkyl groups were radioisotopes. Analysis of the polar dyes isolated from the liver indicated that: the ethyl group was preferentially cleaved, only one methyl of the dimethyl compound was cleaved, and the dye was not bonded to the liver protein through the N-methyl group (the latter in confirmation of work by Lin et al (1967).

Harris et al (1971) showed that $Me_2NC(O)N = NC(O)NMe_2$, a tetramethylated azodicarbonamide, reversibly converted glutathione (GSH) to the oxidized form (GSSG) in nucleated mammalian cells with only nominal oxidation of protein SH. The authors considered the azo compound a useful tool in the study of the biochemistry of GSH.

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Gangolli et al (1972) demonstrated that the complexes between rat serum protein and the azo food dyes Sunset Yellow FCF, Black PN, and Black 7984 readily separated during electrophoresis on cellulose acetate.

The protein-amaranth complex did not separate on cellulose acetate, but did on polyacrylamide gel.

DuPlooy and Dijkstra (1972) studied the polar extractable metabolites from the liver of rats given 4-dimethylaminoazobenzene. Between one and thirteen hours after dosage six metabolites were present at any one time, but at the fourth hour the amount peaked and consisted mainly of the O-sulfate esters of 4'-hydroxy-4-methylaminoazobenzene and 4'-hydroxy-4-dimethylaminoazobenzene. If at the time of dosing an i.p. dose of methionine-S-35, or if 1/4-1/2 hour before sacrifice a s.c. dose of ionic sulfate-S-35, were given, then S-35 was incorporated into the metabolites.

Henkens and Sturtevant (1972) reported that the esterase activity of bovine carbonic anhydrase was completely inhibited by the metal-chelator 4-(8-hydroxy-5-quinolylazo)-1-naphthalenesulfonate.

Connors et al (1972) compared the ability of various preparations from rats to reductively cleave the azo bond of 2'-carboxy-4-di(2-chloroethyl)amino-2-methylazobenzene. The preparations were supernatants from homogenates of gut, gut (entire homogenate), Walker 256 tumor cells, spleen, kidney, and liver. The relative rates of cleavage, in the same order, were: 1, 4.4, 5.3, 7.9, 8.1, and 27.

Albrecht et al (1973) compared the effects on liver functions of medium term feeding to rats of amaranth and 4-dimethylaminoazobenzene. The animals were fed from weaning, up to 2, 3, 4, and 9 months of age, consuming over these periods (in grams): 1.4 or 0.6, 3 or 1.2, 16 or 1.9, 43 or 5.1 of amaranth or the carcinogen, respectively. The amaranth had no effect on weight gain or liver weight (as a percentage of body weight). The carcinogen caused a noticeably lower weight gain at 2 months, but at 9 months the controls were only slightly heavier. The

carcinogen caused liver weights to be much higher as a percentage of body weight. The amaranth had no effect on the percentage of protein in the liver, while the carcinogen caused reductions at 2 and 9, but not 3 and 4 months. The amaranth had no effect on glucose-6-phosphatase, while the carcinogen lowered it at 2, 4, and 9 months. The amaranth had no effect on glucose-6-phosphate dehydrogenase, while the carcinogen raised it at 2 and 9 months. The amaranth had no effect on 6-phosphogluconate dehydrogenase until 9 months, when a lowering occurred; the carcinogen, on the other hand, raised this enzyme level at 2 and 9 months. Amaranth had no effect on the ability to cleave the azo bond of amaranth, while the carcinogen increased this ability at 2, 4, and 9 months (on a per 100 mg of protein basis). Neither dye had any effect on the activity of NADPH-cytochrome C reductase. Liver homogenate supernatant was used for the enzyme studies.

Chauveau and Benoit (1973) fed weaned rats a diet containing 0.06% 4-dimethylaminoazobenzene (DAB) or 0.063% 2-methyl-4-dimethylaminoazobenzene (2-Me-DAB) for 1-3 weeks. DAB bound to total liver protein was the same at 14 as 7 days, then increased 20% at 21 days. Contrastingly, 2-Me-DAB bound to total liver protein was the same as DAB at 7 days, but increased steadily (by 57% at 21 days). DAB bound to liver DNA peaked at 14 days, then dropped by more than 50% at 21 days. The 2-Me-DAB bound to liver DNA peaked at 7 days and remained unchanged. The DNA-bound DAB/2-Me-DAB ratio was 2.47, 3.69, and 1.6 at 7, 14, and 21 days, respectively.

VIII. BIOLOGY

A. Metabolic effects

1. absorption

Radomski and Mellinger (1962) found only 2-4% absorption by rats of the food dyes amaranth, Ponceau SX, and Sunset Yellow from the g.i.tract.

Ryan and Welling (1967) found that chemically pure Sudan III fed to rats as a suspension in methyl cellulose mucilage, olive oil, or oleic acid showed negligible absorption from the g.i. tract. Previous reports to the contrary presumably resulted from the use of impure commercial material.

Walker (1970) reviewed the literature on metabolism of azo compounds, including absorption. His review indicates that in general, highly sufonated dyes aren't absorbed. However, there is a good possiblity that they may be cleaved at the azo bond by the intestinal flora, and the metabolites may be absorbed. The same "pre-metabolism" interferes with judgments on the extent of absorption of the non-sulfonated, oil soluble dyes.

Gibaldi and Grundhofer (1972) found that the permeability of everted rat small intestine to methyl orange increased 100% after a 1/2 hour contact period.

2. excretion

MacDonald et al (1953) studied the metabolism of the N-methyl groups of strongly and weakly carcinogenic members of the N- and ring-methylated-4-aminoazobenzene series. Using C-14 labeled N-methyl groups for ease of detection, they reported the results in Table 16. Prefeeding of the appropriate dye (non-labeled) for three weeks prior to the gastric tube dosing with the labeled sample (+ in the second column) had no consistent effects on the excretory pattern. No discernible pattern correlating

TABLE 16.

DISTRIBUTION OF C^{14} IN THE RESPIRATORY CARBON DIOXIDE, URINE, AND FECES OF RATS FED CERTAIN N-METHYL-C^{14}-LABELED DYES

N-Methyl-C^{14}-labeled dye	Prefeeding of unlabeled dye	Per cent of total activity administered: Expired CO_2 5 hr.	Expired CO_2 10 hr.	Expired CO_2 48 hr.	Feces 48 hr.	Urine 48 hr.	Accounted for in 48 hr.
DAB	−	25	44	60	6	14	80
	+	25	51	66	4	10	86
MAB	−	25	38	48	5	18	71
	+	21	40	51	4	18	74
3'-Methyl-DAB	−	24	47	66	7	14	87
	+	23	42	56	5	21	82
4'-Methyl-DAB	−	21	35	46	14	17	77
	+	20	33	47	6	16	69
4'-Methyl-MAB	−	23	42	60	7	23	90
	+	22	37	49	8	35	92
3-Methyl-MAB	−	24	40	60	6	10	76
	+	20	38	59	9	14	82

carcinogenicity or degree of N-methylation with distribution of excreted C-14 was found.

Ishidate and Hashimoto (1959) examined the urine of dogs after oral dosing with 4-aminoazobenzene (AB), 4-dimethylaminoazobenzene (DAB), or 4'-hydroxy-4-aminoazobenzene. In the 3-7 hour urine was found AB, N-methyl AB, 3-$KOSO_3$-AB, 4'-$KOSO_3$-AB, 3-$HOSO_3$-AB, 3-$HOSO_3$-N-glucosiduronate-AB (probably). In the 24 hour urine was found o- and p-hydroxyaniline, toluene, p-phenylenediamine, AB, 4-methyl AB; the o-hydroxyaniline predominated.

Ryan and Wright (1961) examined the biliary excretion of unchanged dye after i.v. injection of a number of food quality dyes in rats. Their results are given in Table 16a, and do not indicate any relationship between structural type and metabolization.

TABLE 16a.

BILIARY EXCRETION OF WATER SOLUBLE SULPHONATED AZO DYES FROM RATS

Name	Colour Index No.	Average excreted per cent (4 exp.)	Excretion range
Azobenzenes			
Methyl orange	13025	55	38–59
3-Sulpho-4-dimethylaminoazobenzene	—	27	15–40
Fast yellow	13015	10	2–16
Phenylazonaphthalenes			
Naphthalene fast orange 2GS	15510	46	25–60
Red 10BS	17200	12	5–20
Geranine 2GS	18050	64	60–70
Ponceau RS	16150	15	10–20
Orange GCN	15980	23	10–40
Sunset yellow	15985	22	20–30
Scarlet GN	14815	0	—
Ponceau SX	14700	48	30–60
Azonaphthalenes			
Carmoisine	14720	38	30–40
Brilliant scarlet	16255	34	30–45
Amaranth	16185	53	43–79
Phenylazopyrazoles			
Tartrazine	19140	1	0–2
Lissamine fast yellow 2G	18965	96	95–100

Radomski (1961) studied the fate of Citrus Red No. 2 and external D & C Red No. 14 when administered to various animals. Rats excreted unchanged No. 2 (stomach tube administration) in the feces within 48 hours: 6 ± 1% of a 2-20 mg dose, 0% of a 0.5 mg dose. However, they excreted 26% of a 15 mg dose administered as 1/2% of the normal diet. Rabbits excreted 0.9% of a 200 mg tubal dose, 0% of a 100 mg dose. Dogs excreted 1.1% of a 100 mg tubal dose, 0% of a 20 mg dose. Very similar results were found with No. 14 dosage (no normal diet test was done).

Dogs given a tubal 100 mg dose of No. 2 did not excrete unchanged dye in the bile. No test of No. 14 was made.

Glucuronide analysis on 24-hour urine of three each male and female rats given 100 mg/kg tubal No. 2 showed increases of 2.4-7.8 fold. Ethereal sulfate analysis on the same urine (pooled) showed a 2.1 fold increase. The major component in the urine was the O- glucuronide of the unchanged dye. Of the total of eight suspected dye related components found in the urine, the only other positive identification was 1-amino-2-naphthyl sulfate. Experimental difficulties prevented confirmation of the O- glucuronide of 1-amino-2-naphthol as another possibility. When No. 14 labeled with C-14 in the naphthalene ring was given orally to rats, it was found that in a

27 day period 86% of excreted activity was in the feces, 14% in the urine. About 43% of the administered dose of activity appeared in the feces within 24 hours, then fell off rapidly. Urinary activity was about the same at 27 days as at 72 hours. There were three more colored metabolites of No. 14 in the urine than No. 2 (2), and a total of nine suspected compounds, the only identification being 1-amino-2-naphthyl sulfate.

The intestinal contents of five rats were removed and mixed with 200-300 μg/g of No. 2. Within 24 hours there was no remaining intact dye in two cases, all of it remaining in two cases, and 23% remaining in the last. The same experiment on dog and rabbit intestines showed rapid dye destruction in all cases. Attempts to measure the fecal excretion of administered dye and then run the in vitro study on the intestine of the same rat showed no correlation between in vivo and in vitro results. The in vitro rat, rabbit, and dog studies with No. 14 gave the same results as with No. 2.

Radomski (1962) in a follow-up study was able to confirm the presence of the O-glucuronide of 1-amino-2-naphthol in the urine of rats given external D&C Red No. 14. He was not able to detect this substance in the urine of dogs similarly dosed.

Radomski and Mellinger (1962) found that in oral dosing of rats with amaranth, Ponceau SX, and Sunset Yellow food dyes, increased quantities of unchanged dye in the feces could be generated by dosing with antibiotics to depress the activity of the intestinal flora. Of the 2-4% of these dyes which was absorbed through a normal g.i. tract, most was excreted unchanged in the bile.

Robinson et al (1964) gave rats i.p. injections of 4-aminoazobenzene (AB), N,N-dimethyl AB, 3',N,N-trimethyl AB, or 2,N,N-trimethyl AB, and

then analyzed the urine at 24, 48, and 72 hours for certain azo cleavage metabolites (the urine was acid hydrolyzed). About 10% of the N,N-dimethyl AB appeared as p-phenylenediamine, and 70% as p-aminophenol within 24 hours; neither increased over the additional 48 hours studied. About 18% of the 3',N,N-trimethyl AB appeared as p-phenylenediamine, and 28% as 4-amino-2-methylphenol within 24 hours; neither increased over the additional 48 hours. About 18% of the AB appeared as p-phenylenediamine, and 90% as p-aminophenol within 24 hours; neither increased over the additional 48 hours. Only the 2,N,N-trimethyl AB showed increases with time; one metabolite, 2,5-diaminotoluene rose from 14 to 37 to 78%, while the other metabolite, p-aminophenol, rose from 47 to 73 to 80% of the theoretical. The separate injection of p-phenylenediamine resulted in enough more of it being excreted in the urine to indicate that the low amounts found above didn't likely result from in situ destruction.

Radomski and Harrow (1966) administered 1-(o-tolylazo)-2-naphthylamine (Yellow OB) into the stomachs (ligated just beyond the pyloric sphincter) of rats. After six hours they were able to extract material corresponding in UV spectrum to an imidazole, resultant from the reaction of an aldehyde with the dye. Separately the authors administered a single dose of C-14 labeled dye to rats and examined the feces and urine for four days. Of the total activity excreted, 87.5% appeared in the initial two days; of this 82.2% was in the feces and 17.8% in the urine. Chromatography of the feces extracts revealed unchanged dye, and dye with a hydroxy at the 6 position of the naphthyl. In still another test the rats were given the dye orally at the same time an i.m. dose of S-35 sulfate was given; both bile and urine were collected. In the bile were found six colored and one colorless metabolites: unidentified (S-35), unidentified (no S-35), 1-(o-tolylazo)-

6-hydroxy-2-naphthylamine-O-hydrogen sulfate N-glucuronide (S-35), 1-(o-tolylazo)-6-hydroxy-2-sulfaminonaphthalene-O-glucuronide (S-35), 1-(o-tolylazo)-6-hydroxy-2-naphthylamine-N-glucuronide (no S-35), 1-(o-tolylazo)-2-sulfaminonaphthalene (S-35), and a naphthalene derivative of reduced azo (colorless, no S-35). In the urine were found three colored and three colorless metabolites: unidentified (S-35), 1-(o-tolylazo)-6-hydroxy-2-sulfaminonaphthalene-O-glucuronide (S-35, also found in bile), 1-(o-tolylazo)-2-sulfaminonaphthalene (S-35, also found in bile), unidentified (colorless, no S-35), unidentified (colorless, no S-35), and unidentified (colorless, S-35).

Sato et al (1966) investigated the occurrence of N-hydroxy metabolites in the urine of laboratory test animals given parenteral doses of carcinogenic and non-carcinogenic members of the 4-aminoazobenzene family. Their findings are given in Table 17. The authors were able to synthesize N-hydroxy-4-aminoazobenzene, but found it somewhat unstable even when kept at 0-5°C under a nitrogen atmosphere.

Ryan and Welling (1967) gave a single oral dose of pure Sudan III, or a single i.p. dose of pure Sudan III and Sudan IV to rats. There was no excretion of either, or any metabolites, via the bile or urine. From the oral dose of III, 84-95% was recovered in the feces, unchanged. From the i.p. dose of III, 6% or less, depending on amount given, was recovered in the feces, unchanged. From the i.p. dose of IV, less than 3.5% was recovered in the feces unchanged. When an i.p. dose of III tritiated in the terminal benzene ring was given, after 96 hours only 16% of the activity had appeared in the urine (only 9% in a female), 5% in the feces (2% female), and < 1/2% in the bile. The major metabolite (80%) in the urine proved to be 4-aminophenol. The authors commented that the failure

TABLE 17.

THE URINARY EXCRETION OF *N*-ACETYL-4-AMINOAZOBENZENE AND ITS *N*-, 3-, AND 4'-HYDROXY DERIVATIVES BY RATS, MICE, AND HAMSTERS AFTER ADMINISTRATION OF AMINOAZO DYES[a]

SPECIES	SEX	COMPOUND INJECTED AND ROUTE[b]	DIET	NO. OF ANALYSES[c]	% OF DOSE EXCRETED AS			
					AAB	*N*-Hydroxy-AAB	3-Hydroxy-AAB	4'-Hydroxy-AAB
Rat	F	AAB (s.c.)	Control	1 (8)	0.31	0.30	0.60	0.70
	M	AAB (i.p.)	Control	7 (41)	0.18 ± 0.07[d]	0.25 ± 0.08	0.33 ± 0.17	[illegible]
	M	AAB (i.p.)	Ribo.-def.	1 (8)	0.11	0.22	0.30	[illegible]
	M	*N*-Hydroxy-AAB (i.p.)	Control	2 (7)	0.17	0.92	0.21	[illegible]
	M	*N*-Hydroxy-AAB (i.p.)	Ribo.-def.	1 (1)	0.71	0.11	0.28	0.17
	M	AB (i.p.)	Control	1 (1)	0.09	0.02	0.08	n.i.[e]
	M	AB (i.p.)	Ribo.-def.	1 (10)	0.07	0.10	0.17	0.04
	M	MAB (i.p.)	Control	1 (6)	0.04	n.i.	0.02	n.i.
	M	MAB (i.p.)	Ribo.-def.	1 (8)	0.07	0.06	0.13	[illegible]
	M	DAB (i.p.)	Ribo.-def.	1 (8)	0.03	0.02	0.06	0.04
	M	*N*-Hydroxy-AB (s.c.)	Control	1 (6)	0.03	0.17	0.15	n.i.
Mouse	F	AAB (i.p.)	Control	2 (16)	0.69	2.4	0.10	0.80
	F	*N*-Hydroxy-AAB (i.p.)	Control	1 (6)	0.80	21.0	0.12	[illegible]
	F	AB (i.p.)	Control	1 (10)	n.i.	0.12	n.i.	0.04
	F	*N*-Hydroxy-AB (s.c.)	Control	1 (6)	n.i.	n.i.	n.i.	n.i.
Hamster	M	AAB (i.p.)	Control	1 (4)	0.04	2.3	0.13	1.1
	M	*N*-Hydroxy-AAB (i.p.)	Control	1 (2)	0.15	5.2	0.18	1.6
	M	AB (i.p.)	Control	1 (8)	0.10	2.1	0.32	0.84

[a] The abbreviations used are: AB, 4-aminoazobenzene; MAB, *N*-methyl-4-aminoazobenzene; DAB, *N*,*N*-dimethyl-4-aminoazobenzene; AAB, *N*-acetyl-4-aminoazobenzene; ribo.-def., riboflavin-deficient.

[b] Injection schedules: *N*-Hydroxy-AB was dissolved in tricaprylin (17.8 mg/ml), and 0.33 or 0.20 ml was injected s.c. into rats or mice, respectively. The other male rats were injected i.p. at 0 and 6 hr with an amount of dye equimolar to 10 mg of AAB/100 g body weight; 20.5 mg of AAB or an equivalent amount of another dye were suspended per ml of 0.9% sodium chloride solution. The female rats received 10 mg of AAB in 0.2 ml of tricaprylin. The mice were injected once with 0.2 ml of a tricaprylin suspension (equivalent to 10 mg of AAB/ml), while the hamsters received 0.25 ml of a tricaprylin suspension which contained 5.0 mg of AAB or equivalent amount of AB or *N*-hydroxy-AAB/ml.

[c] The numbers in parentheses denote the No. of animals whose urine was pooled for the analyses.

[d] Average ± the probable error.

[e] n.i. (not identified) signifies that no metabolite could be identified. The total absorption in these areas generally accounted for less than 0.01% of the dye administered. In the case of mice administered either AB or *N*-hydroxy-AB, however, an unidentified metabolite was found on the chromatograms in the position normally occupied by AAB, and no AAB could be detected. In these cases the unidentified metabolite may have accounted for about 0.1% of the administered compound.

to find this metabolite in the urine of rats dosed with unlabeled dye resulted from the very low quantity involved.

Fore et al (1967) found that Brown FK (a complex mixture) was only decolorized (azo linkages reduced or broken) by the contents of rat caecum or distal small intestine, and not by stomach or proximal small intestine contents. Sulfanilic acid and a material similar to aminophenazines (producable from polyaminobenzenes by condensation-oxidation) were

recovered from these in vitro experiments. The accumulation of the breakdown products inhibited further breakdown of the components of the dye. In Table 18 are the results of i.p. and intragastric dosing of rats with regard to appearance in caecum, feces, and urine of unchanged components of the dye (A and B bands), sulfanilic acid, phenazine-like material, etc. Only traces of the phenazine-like material were actually excreted after intragastric dosing, the ultimate fate after creation in the caecum being unknown. Similar results derived from daily oral dosing of pigs for three months with 100, 250, and 500 mg/kg of Brown FK; traces of the phenazine material were found in the intestines at the two higher dosages. With rabbits given 1-9 daily doses of 1 g/kg the phenazine material showed up only in the urine, and in more than trace amounts; otherwise results were similar to those from rats and pigs. With guinea pigs given 3-11 daily doses of 1 g/kg, the phenazine material showed up in the caecum. Prior to the experiment with pigs the authors would not have expected the finding of sulfanilic acid and the phenazine-like material in rats to have had much meaning relative to the toxicity of Brown FK in humans. However, with this finding they considered it of importance to determine just which bacteria were responsible and how widespread, interspecially, they might be.

Hanaki (1967) reported that rats fed N-methyl-N-isopropyl-4-aminoazobenzene excreted 4-aminoazobenzene and N-isopropyl-4-aminoazobenzene in the bile and urine. Also, by incubating the N,N-diallyl parent with rat liver homogenate, the methyl group was the one to be cleaved. However, the polar dye isolatable from the rat liver was shown to be N-methyl-4-aminoazobenzene.

Walker (1970) had a good review of the literature, inclusive of excretion, into 1969.

Table 18. *Chromatographic findings* with urine, faeces and caecal contents† of rats given multiple doses‡ of Brown FK*

Specimen	Principal findings	Route	Intragastric						Intraperitoneal					Level in diet (%) (1-, 3- & 12-wk results)			
		Dose (mg/kg/day)	100			1000			100	500			1000	0·001	0·01	0·1	1·0
		Interval after last dose(hr)	0–3	0–6	18–24	0–3	0–6	18–24	18–24	0–3	0–6	18–24	18–24	0–6	0–6	0–6	0–6
Urine	Brown FK (B band)		0	0		Tr	Tr		Tr	0–+++	+++	0		0	Tr	Tr	+
	'Brown colour'		Tr–+	Tr–+	Tr	Tr–+	+	Tr	Tr	0–++	++	0	++	0	Tr	Tr	+
	Sulphanilic acid		+	+	+	+–+++	+++	+++	0	0–+	+	+	+	0	Tr	+	++
	'Blue material'		0§	0§	Tr	0§	Tr–+§	+++	Tr	0	0	0	+	0	0	0	+
	UV-fluorescent spots		1	1		1–2	1–2	1–2		1	2	1		0	Tr	+	+
	Yellow spot (ahead of A band)									+	+						
Faeces	Brown FK (A band)		0	0	0	0	0	Tr	0	0	0	0	Tr	0	Tr	+–	Tr
	Brown FK (B band)		0–Tr	0	0	0–+	++	+	0	0	0	0	Tr	0	Tr	Tr	+
	Sulphanilic acid		Tr–+	Tr–+	0	+–++++	++	++	0	Tr–+	Tr–+	+	Tr	0	0	Tr	+
	'Conjugated colour'		Tr–+	Tr–+	0	+–+++	+++	+++	0	0–Tr	0	Tr	+	0	0	Tr	+
	'Polymeric material'				Tr			+	0			0	0	0	Tr	Tr	+
	Phenazine-like material (P)		0	0	0	Tr–+	Tr	Tr	0	0	0	0	0	0	0	0	0
	UV-fluorescent spots		Tr–1	Tr		1	1	2		1	1	0					
Caecal contents	Brown FK (A band)		0	0		0	Tr–+++	0		0	0	0					
	Brown FK (B band)		0	0–Tr		+–	+–++	0		0	0	0					
	Sulphanilic acid		+	++		+++	+++	+		+	+	+					
	'Conjugated colour'		Tr–+	++		+++	+++	+		0	0	0					
	'Polymeric material'							Tr				0					
	Phenazine-like material (P)		0–+	0–Tr		++	+–+++	0		0	0	0					
	UV-fluorescent spots		Tr–1	1		1	1	0		2	2	0					

*Symbols used: 0, no difference from controls without Brown FK; Tr, trace; +, ++ and +++ present in small, moderate and large amounts, respectively.
†Caecal contents were obtained at autopsy, carried out at the end of the stated interval after the last dose of Brown FK.
‡A total of 10 daily doses was given by intragastric or intraperitoneal routes.
§Colourless material giving yellow colour with Ehrlich reagent also present in this position.

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Ryan and Welling (1970) dosed rats orally and parenterally, and man orally, with the food dye Black PN. Qualitative findings of the excretory routes of the dye and its metabolites are given in Table 19. The last five columns on the right correspond to: azo metabolite, sulfanilic acid, non-azo metabolite, non-azo metabolite, and non-azo metabolite, respectively (all identified). There are two possible azo metabolites as there are two azo linkages in the dye, but only one, apparently, was found. Residual dye and the azo metabolite were found in the stomach wall and contents. SA and DSA were found in the intestinal contents. None of the azo metabolite or dye was found in the bladder, intestines, heart, liver, or stomach after i.v. dosage. In Tables 20, 21, and 22 are the quantitative measurements of excreted metabolites. It was not found possible to quantitize

Table 19. *Excretion of Black PN and metabolites in rats and man dosed with the colouring*

Species	Route of dosage	Excretory route	Black PN	SNSA	SA	DSA	ANSA	AHNDA
Rat	Oral	Urine	−	−	+	−	+	−
		Faeces	+*	−	+	+	+	+
	Intraperitoneal	Urine	+*	+*	+	+	+	+
		Faeces	−	−	+	+	+	+
	Oral	Bile	+	+				
	Intravenous	Bile	+	+	−		+	
Man	Oral	Urine	−	−	+			

+ = Compound present − = Compound absent

*Found only after single dose of 100 mg/rat.

Table 20. *Quantitative excretion of metabolites of Black PN after a single oral dose of the colouring to rats and man*

Metabolite excreted	Excretory source	Time (hr)	Excretion of metabolite (% of theoretical yield) with Black PN dose of		
			20 mg/rat	100 mg/rat*	240 mg/man
SA	Urine	24	36·7	40·5	23·6
		48	6·9	17·8	13·5
		72	0·9	2·5	
	Total ..		44·5	58·8	37·1
	Faeces	48	29·5	30·2	
		72	5·7	2·8	
	Total ..		35·2	33·0	
	Urine/faeces total.. ..		79·7	91·8	
ANSA	Urine	24		<1·0	
	Faeces	48		<1·0	

*Trace of Black PN in faeces.

Table 21. *Quantitative estimation of some metabolites in bile and the gastro-intestinal tract 8 hr after an oral dose of 25 mg Black PN/rat*

Metabolite	Source	Theoretical yield of metabolite (%)
SNSA	Bile	Trace
SNSA	Stomach + contents	3·4
Black PN	do.	18·4
SA	Intestine + contents	10·1

Table 22. *Quantitative excretion of some metabolites of Black PN after intraperitoneal injection of the colouring to rats*

Metabolite excreted	Excretory source	Time (hr)	Excretion of metabolite (% of theoretical yield) with Black PN dose (mg/rat) of		
			10*	20*	100†
SA	Urine	24	60·2	59·4	45·7
		48	8 0	5·9	15·7
		72	0 0	0·0	3·5
		Total ..	68·2	65·3	65·9
	Faeces	48	13·9	20·1	19·0
		72	6·7	4·0	5·9
		Total ..	20·6	24·1	24·9
	Urine/faeces total		88·8	89·4	90 8
Black PN	Urine	24	0·0	0·0	0 6
SNSA	Urine	24	7·7	8·2	6·0
ANSA	Urine	24	5·0	2·0	—

*No Black PN in urine or faeces; no SNSA in faeces.
†No Black PN or SNSA in faeces.

the metabolites DSA and AHNDA because of their chemical instability once isolated. Production of sulfanilic acid apparently only occurred in the intestine, as a result of bacterial action. The rat's enzymes did not seem able to reduce the azo bond which would result in this particular metabolite, but they did reduce the other azo link.

Gingell et al (1971) investigated the influence of intestinal bacteria on the reductive cleavage of the azo bonds of Prontosil and Neoprontosil by treating rats with antibiotics prior and subsequent to dosing with the azo compounds. In Table 23 is the comparison between control and antibiotic treated animals with regard to excretion in urine and feces, and nature of the metabolites, showing the strong effect of the treatments. In Table 24 it is seen that, with Neoprontosil, the bacteria are involved even when

the dye is introduced i.p. When biliary-cannulated rats were given an i.p. dose of the S-35 Prontosil, 48% of the dose was excreted in the bile after 48 hours as the glucuronide, and only 3% as sulfanilamide; when the dose was oral, these figures were 23.5 and 1.9%, respectively. When Neoprontosil was dosed orally to these altered rats, 1.4% of the glucuronide appeared in the bile after 24 hours, and 14% appeared as sulfanilamide in the urine at the same time; when the dose was i.p., these figures were 65 and 14%, respectively; when the dose was i.v., these figures were 67 and 9%, respectively.

3. transport

Ryan and Welling (1967) showed that unchanged dye could be recovered in the feces of rats given i.p. doses of the dyes Sudan III and IV. Since there was no biliary excretion of the unchanged dye, there must have been diffusion through the peritoneum and intestinal wall.

Ozkan (1970) showed that 3'-methyl-4-dimethylaminoazobenzene could be transported across the placenta in rats, producing changes in the liver of the offspring in line with the amount of dye ingested by the parent.

Golub (1971) demonstrated that o- and p-aminoazotoluene could be transferred across mouse placenta.

4. distribution

MacDonald et al (1953) fed rats N-methylated (C-14)-aminoazobenzenes and measured the distribution of radioactivity in the g.i. tract and in liver proteins - Table 24; in the table MAB and DAB are abbreviations for 4-methylaminoazobenzene and 4-dimethylaminoazobenzene.

Table 23. The distribution of ^{35}S in rats receiving [^{35}S] Prontosil orally with and without treatment with antibiotics

The oral dose of [^{35}S] Prontosil hydrochloride was 56 mg/kg and each rat received 10 μCi of ^{35}S. The antibiotic treated rats each received orally neomycin sulphate (100 mg), bacitracin (50 mg) and tetracycline hydrochloride (50 mg) twice daily for two days before Prontosil. The antibiotics were then given 4 h before and 4 and 24 h after the administration of Prontosil. The urinary metabolites were separated by paper chromatography and radiochromatogram scans prepared. The metabolites were determined by cutting out the appropriate areas from the paper and counting the areas in the scintillation spectrometer. The figures given are the average values for three rats with ranges in parentheses.

Material examined	Days after dosing	% Dose of ^{35}S found in Control rats	% Dose of ^{35}S found in Antibiotic-treated rats
Urine	2	81(78–84)	43(41–45)
Faeces*	2	2·1(1·7–2·4)	4·4(1·7–6·2)
Liver + lung + kidneys + spleen	2	1·8(0·4–2·9)	1·8(1·0–3·4)
Gastointestinal tract + contents	2	3·3(2·2–4·3)	36(30–45)
Rest of carcass	2	2·4(2·3–2·4)	6·9(5·4–9·6)
Total of above items	2	88(84–92)	92(90–96)
Components of urine			
Prontosil *N*-glucuronide	0–1	4·6(3·5–5·6)	1(1–1)
	1–2	0·8(0·6–0·9)	3(2–3)
	Total	5·4(4·3–6·2)	4(3–4)
Total sulphanilamide†	0–1	60(52–71)	6(5–6)
	1–2	14(8–21)	33(31–35)
	Total	74(72–79)	39(35–41)
Sum of above components	Total	79(76–85)	43(39–45)

* At least 80% of the faecal activity was present as sulphanilamide (free + acetylated).

† Free + acetylated: in the control rats about 82% and in the treated rats about 90% of the total sulphanilamide excreted was acetylated.

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Table 24. The effect of antibiotics on the excretion of total sulphanilamide by rats receiving Neoprontosil

Neoprontosil disodium salt (100 mg/kg) was administered dissolved in water. The antibiotic-treated animals received orally 100 mg of neomycin sulphate and 30 mg of nystatin twice daily for 2 days before and after giving the drug. Urine was collected daily and analysed for free and total sulphanilamide. The results are the averages for three animals with ranges in parentheses.

Route of administration	Treatment	Days after dosing	% Dose excreted in urine as total sulphanilamide*
Oral	None	1	42(38–45)
		2	43(41–45)
Oral	Antibiotics	1	13(9–15)
		2	17(10–24)
Intraperitoneal	None	1	31(26–39)†
		2	37(27–41)
Intraperitoneal	Antibiotics	1	19(11–26)†
		2	26(18–36)

* Free + acetylated; in this series of experiments the extent of acetylation was 77–86% of the total sulphanilamide excreted and was unaffected by the antibiotics.

† Some unchanged Neoprontosil (0·10% of dose) was found in the urine after i.p. injection.

TABLE 24a.

DISTRIBUTION OF RADIOACTIVITY IN RATS FED CERTAIN N-METHYL-C^{14}-LABELED DYES

	DAB					4'-methyl-DAB		3-methyl-MAB		3'-methyl-DAB	
	M	M	F	F	F*	M	M	M	M	M	M
Duration of experiments, hrs.	5	10	10	10	10	5	10	5	10	5	10
Per cent total activity:											
In respired CO^2, 5 hrs.	26	28	25	25	19	23	20	27	18	15	29
" , 10 "		46	47	48	36		40		41		54
In stomach and small intestine contents	13	8	5	3	8	20	8	12	7	27	5
In cecum and large intestine and feces	5	8	6	2	5	3	6	4	4	1	6
Standard specific activity† of:											
Liver protein	100	140	250	200		80	150	100	150	150	100
Liver serine (protein-bound)	1,000	1,400	3,400	2,300		750	2,000	530	950	950	1,600
Liver choline	4,600	5,000	7,200	6,000	9,400	2,800	5,300	4,900	4,600	3,100	5,700
Per cent activity:											
Of serine in β-carbon	98	104	100	100		99	95	93	94	93	99
Of choline in methyl groups	66	77	70	77	68	100	76	89	84	82	77

* Pooled samples from two immature rats; see "Methods."

† Standard specific activity (counts/min/mg) = $\frac{10^2 \times \text{observed specific activity}}{\text{activity administered (counts/min)}}$

Berenbom (1959) fed male rats for four weeks on a diet containing 0.06% 4-dimethylaminoazobenzene (DAB), then for 4-5 days using N-15 labeled DAB. In separate experiments the labeled N was as follows: C_6H_5-N* = N-$C_6H_4NMe_2$ (DAB-1), C_6H_5-N = N*-$C_6H_4NMe_2$ (DAB-2), and C_6H_5-N = N-C_6H_4N*Me_2 (DAB-3). The rats were sacrificed and the homogenized livers centrifuged into nuclear, mitochondrial, and microsome-supernatant. In a different series of otherwise identical feedings, the three fractions were further broken apart by solvent extraction. Tables 25, 26, 27, and 28 present the findings on the distribution in the liver of metabolites of DAB.

Baba (1961) gave a rat 15 mg of DAB (C-14 labelled in the non-amino ring) via stomach tube on two occasions in a single day, followed by a rice diet containing 0.06% of the C-14 DAB for 72 hours (elapsed time from the

initial force feeding). The liver was sectioned, rinsed free of non-protein bound DAB, and then autoradiographed. Very little activity appeared around the bile ducts. Distribution of the activity was nearly uniform across the peripheral, middle, and central zones, slightly lower in the peripheral. Activity in the two lobes was the same. Slight activity existed in Kupffer's cells.

Radomski (1961) gave stomach tube doses of Citrus Red No. 2 to male and female rats. After 24 hours no unchanged dye could be found in kidney, liver, muscle, or spleen tissue. When the dose was 5 mg, there was no dye in the fat, but there was 4-10 μg/g after a 20 mg dose. Starting with sixteen rats, killing four after one day and three on succeeding days, Radomski gave daily tubal doses of 150 mg/kg (100 mg/kg in a seven day experiment) of the dye. He found that dye content in the fat dropped in a linear fashion at the higher dose - none left on the sixth day from 15 μg/g after the first day, in a logarithmic fashion at the lower dose - none left on the seventh day from 13 μg/g after the first day. This study was repeated on external D&C Red No. 14 with similar results. More of the dye was initially incorporated into the fat than with No. 2. Females showing 13-39 μg/g after dose 1 showed 0 μg/g after dose 7; males showing 13-47 μg/g after dose 1 showed 0 μg/g after dose 10 (6.5-24 μg/g after dose 7). Drop off was smooth in the females, zig-zag in the males.

Storey (1968) studied the distribution and retention in connective tissues and bones of chlorazol fast pink and related dis- and trisazo dyes (i.p. dosage). Two consecutive daily doses of the fast pink at 25 mg/kg to rats resulted in noticeable external coloration still obvious after six months. There was no staining of 17-18 day fetuses. Noticeable color in the urine persisted six months. Internally after one day staining

was obvious in skin, fascia, muscle attachments, most internal organs, aortas, and bone marrow. Also colored was the cartilage in the nose, ear, and trachea. No staining of the brain, spinal cord, eyes, nails, or hair occurred. At six months coloring had become faint except for the aortas, which were still bright. Young, but not old animals showed staining at the margins of cranial sutures, dentine, and long bone growing metaphyses.

Gingell et al (1971) examined the distribution in the bodies of rats after oral dosing with Prontosil S-35. The results have already been displayed in Table 23 in connection with excretion of metabolites. Treatment with antibiotics to study the effect on in-gut breakdown did not change the accumulation in the internal organs, but it did increase the amount in the carcass by a factor of 2.5-4.

TABLE 25.

QUANTITATIVE REFERENCE DATA FOR RATS USED IN N^{15}-LABELED DAB EXPERIMENTS

Series	DAB	Av. body wt. (gm.)	Av. liver wt. (gm.)	Total N in liver (meq/gm wet wt.)	Per cent of total nitrogen			Per cent of total succinic dehydrogenase		
					N	M	S	N	M	S
I*	1	155	8 8	1 39	13	28	59	3	94	3
	2	105	5 4	1 21	18	27	55	4	85	11
	3	125	7 1	1 07	12	28	60	2	93	5
II†	1	106	4.8	1.89	14	25	61	3	90	7
	2	122	5.9	1.94	18	27	55	4	86	10
	3	136	7.1	1 94	12	28	60	3	90	7

* Analysis of individual rat livers; average of three experiments for each sample of DAB.
† Analysis of pool of three to four rat livers; average of three experiments for each sample of DAB.
N = nuclei, M = mitochondria, S = microsome-supernatant.

TABLE 26.

DISTRIBUTION OF N^{15} IN THE NUCLEAR, MITOCHONDRIAL AND MICROSOME-SUPERNATANT FRACTIONS OF THE LIVER OF RATS FED N^{15}-LABELED DAB

Experiment (series I)	N^{15} concentration atom per cent excess $\times 10^3$			Per cent of total N^{15} in liver		
	N	M	S	N	M	S
DAB-1	1 4	1 9	2 9	8	22	70
DAB-2	1 9	3 5	4 1	10	27	63
DAB-3	2.1	2 9	3.7	8	24	68

Each value is the average of three experiments.
N = nuclei, M = mitochondria, S = microsome-supernatant.
The N^{15} concentration has been corrected for the natural content of N^{15} of the particular fractions as determined on rats fed the diet containing nonisotopic DAB.

TABLE 27.

RELATIVE CONCENTRATION OF N^{15} IN THE NUCLEAR MITOCHONDRIAL, AND MICROSOME-SUPERNATANT FRACTIONS OF THE LIVER OF RATS FED N^{15} LABELED DAB

EXPERIMENT (SERIES I)	RELATIVE N^{15} CONCENTRATION					
	N	M	S	N	M	S
DAB-1	1.0	1.4	2.1	1.0	1.0	1.0
DAB-2	1.0	1.8	2.2	1.4	1.8	1.4
DAB-3	1.0	1.4	1.8	1.5	1.5	1.3

N = nuclei, M = mitochondria, S = microsome-supernatant.

TABLE 28.

DISTRIBUTION OF NITROGEN AND N^{15} IN CHEMICAL CONSTITUENTS OF NUCLEAR, MITOCHONDRIAL, AND SUPERNATANT FRACTIONS OF THE LIVER OF RATS FED N^{15}-LABELED DAB (SERIES II)

MATERIAL	TOTAL NITROGEN, MEQ/GM. WET WT. OF LIVER $\times 10^2$			ATOM PER CENT EXCESS $N^{15} \times 10^3$		
	DAB-1	DAB-2	DAB-3	DAB-1	DAB-2	DAB-3
Nuclei:						
Cold acid-soluble	0.8	0.9	0.8	3.1	4.0	4.0
Lipide	9.7	8.0	10.3	2.0	5.8	4.0
Nucleic acid	6.4	7.4	6.7	0*	0*	0*
Protein	11.4	16.4	7.0	1.6	2.0	3.0
Mitochondria:						
Cold acid-soluble	1.0	1.4	1.4	2.7	4.0	4.1
Lipide	11.6	13.7	10.8	2.6	3.2	3.9
Nucleic acid	2.7	3.2	3.2	2.8	3.7	3.9
Protein	28.4	33.3	31.2	1.9	2.6	2.5
Microsome-supernatant:						
Cold acid-soluble	10.4	10.4	8.8	2.4	3.3	4.0
Lipide	35.7	34.7	35.0	2.8	3.6	3.8
Nucleic acid	5.9	6.9	7.4	3.2	3.8	4.0
Protein	67.3	57.1	62.8	2.0	2.6	2.9

* Too low to measure accurately.

The N^{15} concentration has been corrected for the natural content of N^{15} of the particular fractions as determined on rats fed the diet containing nonisotopic DAB.

Tables 25-28 reprinted with permission from Cancer Research 19:1045-49 (1959).

B. Physiological effects

Danneberg and Schmähl (1952) tested the estrus-inhibiting properties in rats of a number of azo compounds. The compounds which had this property were not necessarily also carcinogenic. Strong estrus inhibitors were: 4-aminoazobenzene (AB), N,N-dimethyl AB, N-acetyl AB, 4,N,N-trimethyl AB, 2',3-dimethyl AB. Non estrus inhibitors were: 4-methoxy-N,N-dimethyl AB, 4-nitro-N,N-dimethyl AB, 4-(phenylazo)-N,N-dimethyl AB, 1-(4-dimethylamino-phenylazo)-2-naphthylamine, 1-phenylazo-2-naphthylamine, 1-(2-methylphenylazo)-2-naphthylamine, 1-(2-methoxyphenylazo)-2-naphthol, and 2,4-dihydroxyazo-benzene (4,4'-dihydroxyazobenzene is an estrogen).

Lacassagne et al (1952) fed adult rats four derivatives of azobenzene as 0.06% of their diet until death occurred. All four caused considerable weight loss. In order of decreasing damage to the liver, the compounds were: 3',N,N-trimethyl-4-aminoazobenzene, 4'-N,N-trimethyl-4-aminoazobenzene, 4'-phenyl-N,N-dimethyl-4-aminoazobenzene, and 4-hydroxyazobenzene.

Adams and Roe (1953) applied to the skin or injected beneath it solutions of azo compounds to study the effect on hepatic catalase activity. None of the compounds caused any damage to the liver itself. In decreasing order of ability to depress the enzyme level the compounds tested were: 3',N,N-trimethyl-4-aminoazobenzene, 4-N,N-dimethylaminoazobenzene, 2',3'-dimethyl-4-aminoazobenzene, m-azotoluene, 2-amino-5-azotoluene, and azo-benzene (no depression). Doses applied were 10-15 μm.

Takahashi (1953) reported the following azobenzene compounds to be estrogenic in mice (compound, dose in μg, % of mice responding): 2,2',4,4'-tetrahydroxy, 20, 100 (10, 60); 4,4'-dihydroxy, 1000, 80 (500, 20); 2,4-dihydroxy, 500, 40; 2,4-dihydroxy-2',5'-dimethoxy, 1000, 0.

Reiss et al (1954) gave 80 mg of 3',N,N-trimethyl-4-aminoazobenzene

over a four day period to male and female rats in a vitamin poor diet. In both sexes the liver showed higher B_1 and lower B_2 levels than controls. A similar result obtained with vitamin C, but the females were dependent to some extent on the vitamin content of the pre-experiment diet.

Akai and Yasumori (1955) cultured the fungus Cochliobolus miyabeanus in a nutrient solution containing 10 μM-1 mM Congo Red or 0.1-0.5 mM Chrysoidin. Maximal growth occurred at 0.25 mM Congo Red (39% higher than the control) and at 0.25 mM Chrysoidin (107% higher than the control). At the highest concentrations of both, growth was less than that of the control, the Chrysoidin being the more toxic. Optimal usage of glucose and nitrate occurred at the lowest dye concentration, not at that concentration which gave highest growth. Innoculation of the dye-grown fungus on rice plant leaves showed that there was decreased toxicity to the plant; the decrease was independent of the Chrysoidin concentration, but correlated with the Congo Red concentration (the lower the concentration the higher the toxicity).

Nomura (1955) showed that 2',4,4'-trihydroxy-2-methylazobenzene showed 100% estrogenic activity as a 50 μg s.c. dose in castrated mice, 0% as a 30 μg dose. The same figures applied for 2,2'-dimethyl-4,4'-dihydroxyazobenzene. In the case of 2,4,4'-trihydroxyazobenzene or 2-methyl-4,4'-dihydroxyazobenzene, a 300 μg dose had no effect.

Doi (1957) fed rats 0.06% of 4-N,N-dimethylaminoazobenzene in their diet for 30 days. Examination of the liver showed increases over normal in haemosiderin, ferritin, and ascorbic acid, decreases in catalase, copper, and riboflavin.

Okuda (1959) fed rats a diet containing the usual amount (probably 0.06%) of 4-amino- or 4,N,N-dimethylaminoazobenzene over a month's time

and measured urinary excretion of B vitamins throughout. No reliable change in thiamine resulted. Nicotinic acid increased slightly. Pyridoxic acid decreased noticeably. Riboflavin increased considerably. Guinea pigs did not show the increase in riboflavin.

Neish (1959) gave single i.p. doses of various azobenzenes to female rats, in molar amounts corresponding to 165 mg/kg of 3',N,N-trimethyl-4-aminoazobenzene. Methaemoglobin from tail blood was then determined at 3, 7, 22, and 28 hours. Table 29 sums these four values and presents them as methaemoglobinemia. It may be seen that there was no correlation with carcinogenicity.

Table 29. Methaemoglobinemic Activity of Some Azo Compounds

Azo Dye	Carcinogenicity	Methaemoglobinemia Σ %
Azobenzene	—	0
4-dimethylaminoazobenzene	++	140
4-aminoazobenzene	±	133
4-monomethylaminoazobenzene	++	123
4-monoethylaminoazobenzene	—	126
4-methylethylaminoazobenzene	++	180
2'-methyl-4-dimethylaminoazobenzene	+	38
3'-methyl-4-dimethylaminoazobenzene	+++	53
4'-methyl-4-dimethylaminoazobenzene	±	0
4'-ethyl-4-dimethylaminoazobenzene	+++	0

Mascitelli-Coriandoli (1960) fed rats a diet containing 0.064% 3',N,N-trimethyl-4-aminoazobenzene. After three weeks and six weeks, respectively, the hepatic riboflavin had fallen by 1/3 and 1/2. Corresponding figures for hepatic azoreductase activity reduction were 1/4 and 1/2.

Yamada (1960) gave rats i.p. injections of Trypan Blue once a day for three or six days (A or C), and twice a day for three or six days (B or D). Radio-iodine was given s.c. six hours before autopsy. Thyroidal uptake of the radio-iodine was 55-60% of the control in A, B, and C, but only 14% in D. Thyroid weight ranged 70-80% of normal, D being lowest. Pituitary weight of D was 90% of control. Adrenal weight was 135-155% of

the control, increasing in the order A, B, C, D. Testis weight was 110-115% of control. Total iodine and protein-bound iodine in the serum were 62 (25.0) and 45.8 (25.0)% in C (D), respectively. On average, a single injection of trypan blue inhibited thyroid hormone secretion for 14 hours.

Boyland and Grover (1961) measured urinary ascorbic acid excretion after 100 mg/kg doses of some azo dyes. The greatest percentage increase in excretion resulted from 4',N,N-trimethyl-4-aminoazobenzene, followed by the 2,N,N-, the 3',N,N-, and N,N- itself.

Neish and Rylett (1963) gave rats i.p. injections, 16.5 mg/100 g, of azo dyes, and measured the hepatic glutathione level 24 hours later. The glutathione (dye)/(control) ratios found were: 1.97 for 3',N,N-trimethyl-4-aminoazobenzene, 1.21 for the 4' isomer, and 0.41 for the 2 isomer.

Neish and Rylett (1963, pp. 1147-50), in a follow up report on the effect of 3',N,N-trimethyl-4-aminoazobenzene on rat hepatic glutathione, reported that 24 hours after an i.p. dose of 16.5 mg/100 g the stomach was noticeably dilated and filled with food.

Kizer and Howell (1963) reported a study on the effect of 3'- and 4'-methyl butter yellow on rat hepatic kynurenine hydroxylase activity. Although the control diet seemed to be deficient in something which also affected the enzyme in the same direction as the azo compounds, the latter's effect was still noticeable. The results are in Table 30.

TABLE 30. Effect of a low and a high carcinogenic derivative of 4-dimethylaminoazobenzene on the kynurenine hydroxylase activity of rat liver

Diets	Enzyme activity*			
	0 Weeks	4 Weeks	8 Weeks	12 Weeks
Basal diet	(3) † 0.51 ± 0.04‡	(3) 0.32 ± 0.05	(3) 0.46 ± 0.06	(3) 0.51 ± 0.09
Basal diet with				
0.06% 4'Me-DAB		(3) .14 ± .04	(3) .27 ± .04	(3) .31 ± .10
0.06% 3'Me-DAB		(3) .14 ± .07	(3) .09 ± .03	(3) .19 ± .11

*μMoles 3-hydroxykynurenine formed per mg mitochondrial protein per 90 minutes' incubation.
†Number of experiments; for each experiment, livers from 2 to 3 animals were pooled.
‡Mean ± standard error.

Kline and Clayton (1964) fed rats 0.064% of their diet as 3',N,N-trimethyl-4-aminoazobenzene, and measured the hepatic lactic dehydrogenase activity. The reduction in activity did not become significant until the livers had shown signs of cirrhosis for three weeks. Continuing the feeding until tumors appeared in the liver revealed that the tumors had far less activity than surrounding tissue.

Furlong and Thomann (1964) fed rats 0.06% of their diet as 3',N,N-trimethyl-4-aminoazobenzene and measured the hepatic DNA polymerase. After six days the activity had nearly doubled. By 20 days the activity had peaked at about 2 1/4 times. After seven weeks the activity was only slightly higher than normal.

Dijkstra (1964) gave rats a single intragastric dose of 2,N,N- or 3',N,N-trimethyl-4-aminoazobenzene and measured the hepatic ascorbic acid level. The level remained in the normal range for the initial 40 hours after dosing with the 3', then fell to only 60% of the mean. Between 10 and 30 hours after dosing with the 2 isomer, the level was above the normal range (about 10% higher than the mean); after the 35th hour the level was the same as with the 3' dosage. The levels were still below normal after two weeks.

Dijkstra and Pepler (1964) fed rats diets containing 4-aminoazobenzene (AB), N,N-dimethyl AB, 2,N,N-trimethyl AB, and 3',N,N-trimethyl AB in amounts equivalent to 0.06% of the N,N-dimethyl AB. Over an entire 20 week period hepatic ascorbic acid was above the normal range with the 3'N,N compound, especially from the third week. With the 2,N,N compound the level was at the normal upper limit or slightly above. With the N,N compound problems of interpretation of trend arose because of very wide ranges from the 7th through 14th weeks, but from the fourth week the

level averaged at or above the upper normal. With AB a normal pattern was seen.

Manchon and Lowy (1964) demonstrated that rats fed a diet deficient in vitamin B_2 survived longer and showed better growth when a 2% solution of Sun Yellow in water was their source of water. Omission of all B_2 from the diet resulted in death however.

Mel'nikova and Selikhova (1965) found that mice given azobisformamide produced less serum pseudocholinesterase and acetylcholinesterase, and less hepatic cholinesterase.

Mulay (1966) fed normal, three month old rats a low-protein, low-riboflavin diet containing 0.06% 3',N,N-trimethyl-4-aminoazobenzene for 2, 5, 7, and 17 weeks.

TABLE 31. Effect of Hepatocarcinogenic Diet on Adrenal Gland and Liver Chemistry of Male Osborne-Mendel Rats. Each value is a mean of determinations on 10 rats. Standard error for each value is within 2% of respective mean.

		Adrenal glands				Liver	
Diet	Time, wk	Ascorbic acid, mg/g	Steroids, mg/g	Fat, mg/g	Weight, mg/100 g body wt	Steroids, mg/g	Fat, mg/g
Purina chow (control)		2.38	29.3	186	11.7	3.23	49.3
Hepatocarcinogenic	2	2.51	35.2	198	12.2	3.11	62.4
"	5	3.13	50.5	257	12.6	3.03	70.1
"	7	3.38	61.6	286	12.8	2.97	80.5
"	17	2.17	52.0	290	15.5	3.85	55.7
Semi-synthetic	6	2.98	37.9	194	13.7	4.15	93.7

The results in Table 31 indicate higher steroid and fat content in the adrenals onsetting at 2-5 weeks. The adrenal/body weight ratio was noticeably lower than the deficient diet control through seven weeks. Hepatic steroids and fat were considerably lower than this control. The author claimed that hepatic ascorbic acid and ratio to whole body were the same as controls, but did not specify which controls.

Sydow and Sydow (1967) gave rats a single oral dose of 40 mg of 3',N,N-trimethyl-4-aminoazobenzene and then measured various hepatic functions

over a two month period. There was no change in relative liver size, but hepatic protein dropped about 20% during the first week. The protein level was fully recovered within one month. Hepatic hexokinase was unchanged. The glycogen content dropped by 2/3 in the first day, then gradually recovered, seemingly to a higher than normal level after two months (the authors gave no P values). Glucokinase dropped 2/3 by the fourth day (possibly even lower thereafter) but had recovered after one month. Glucose-6-phosphate dehydrogenase dropped 1/2 by the fourth day, and was nearly, if not actually totally, recovered by one month. Arginase was unchanged.

In a separate experiment the rats were given 5 mg of the azo compound daily for 80-130 days. Relative liver weight was about doubled for 80 and 100 day treatments, but pentupled after 130 days. Hepatic protein dropped 20-25%. Glycogen dropped by 1/3 in the 80 and 100 day treatments, over 2/3's in the 130 day treatment. Hexokinase increased 2-2 1/2 times, but showed a much wider range than the controls. Glucokinase dropped 76% after 80 days, 69% after 100 days (both having relatively high spreads), and 81% after 130 days. Glucose-6-phosphate dehydrogenase dropped by 1/2 regardless of length of treatment. Arginase was unchanged. All of the changes from 80 day treatment were at least partially reversible as seen from 6-8 weeks on a normal diet. After six weeks normal diet after 130 days on treatment relative liver weight was returning to normal, as were the glucokinase and glucose-6-phosphate dehydrogenase activities; on the other hand, hepatic protein, glycogen, and hexokinase showed no change.

Doctor et al (1967) showed that rats fed 0.06% 3',N,N-trimethyl-4-aminoazobenzene showed changes in hepatic and serum vitamin B-12 comparable to those in fasted rats over the initial ten days. Over a 15 week test

period the hepatic level gradually decreased in comparison with controls.

Grasso et al (1968) gave rats either a daily dose of 1 g/kg (by stomach tube) for seven days, or a diet containing 2% for 12 weeks of the British azo food dye Brown FK. Both treatments resulted in myofibrillolysis and lysosomal damage followed by lipofuscin deposition. The lysis is rapid and extensive after the forced feeding, occasionally not appearing after dietary treatment.

Gaunt et al (1968) fed rats Brown FK at 0.001-1.0% of their diet for 21 weeks, and gave miniature pigs daily doses corresponding to 100-500 mg/kg for 24 weeks. Unchanged were growth rate and food consumption, kidney and liver weights and function. Hematology was normal. Apart from the following, histopathology was normal. In the pigs the principal change was deposition of lipofuscin in the liver in both sexes at all dosage levels, accompanied by higher lysosomal enzyme activity. The lipofuscin also deposited in male, but not female, hearts. In the rats the 1% dietary level caused this deposition in the heart, kidney tubules, hepatic Kupffer and parenchymal cells, and skeletal muscle, being noticeable at 13 weeks in females and at the end of treatment in the males.

Gaunt et al (1969) gave pigs 90 daily doses of Ponceau 4R of 100-900 mg/kg. The only effect noted was a slight decrease in erythrocytes in males after six weeks at the highest dose.

Gaunt et al (1969, pp. 557-563) gave pigs 90 daily doses of Black PN of 100-900 mg/kg. The only effect noted was development of mucus and fibrin-containing cysts in the mucosa of the ileum of 1/6 of the 300 and 4/6 of the 900 mg/kg animals.

Beaudoin gave i.p. injections to 8-day pregnant rats of 14 mg/100 g trypan blue, Evans blue, and Niagara blue 4B, and of 20 mg/100 g Niagara

TABLE 32.

Total protein and protein-fraction concentration in serum of control and disazo dye-treated rats expressed as mean values in g per 100 ml with standard deviation

Treatment and day blood drawn	No. females	Total protein	Globulins					Albumin
			Gamma	Beta	Alpha-3	Alpha-2	Alpha-1	
Control								
8	10	6.30 ± 0.43	1.09 ± 0.33	1.09 ± 0.10	0.32 ± 0.04	0.35 ± 0.02	0.78 ± 0.02	2.65 ± 0.36
10		6.25 ± 0.54	0.87 ± 0.26	1.08 ± 0.12	0.32 ± 0.04	0.30 ± 0.06	0.76 ± 0.14	2.86 ± 0.36
20		6.40 ± 0.52	0.49 ± 0.14 *	0.93 ± 0.13 *	0.36 ± 0.06	0.40 ± 0.10	1.54 ± 0.22 *	2.65 ± 0.30
Trypan blue [1]								
8	10	6.40 ± 0.31	0.74 ± 0.10	0.90 ± 0.12	0.40 ± 0.05	0.28 ± 0.06	0.85 ± 0.13	3.23 ± 0.20
10		5.58 ± 0.26 *	0.58 ± 0.10 *	1.06 ± 0.14 *	0.41 ± 0.07	0.43 ± 0.07 *	0.90 ± 0.16	2.20 ± 0.18 *
20		6.73 ± 0.36 *	0.58 ± 0.14	1.01 ± 0.15	0.34 ± 0.04 *	0.28 ± 0.05 *	1.04 ± 0.17 *	3.48 ± 0.34 *
Evans blue [1]								
8	8	6.22 ± 0.16	0.61 ± 0.10	0.88 ± 0.10	0.32 ± 0.06	0.25 ± 0.06	0.78 ± 0.09	3.38 ± 0.24
10		5.83 ± 0.40 *	0.64 ± 0.12	1.29 ± 0.09 *	0.50 ± 0.10 *	0.67 ± 0.04 *	0.94 ± 0.17 *	1.79 ± 0.30 *
20		6.20 ± 0.43	0.38 ± 0.04 *	0.92 ± 0.16 *	0.38 ± 0.13 *	0.34 ± 0.04 *	1.32 ± 0.14 *	2.86 ± 0.31 *
Niagara blue 4B [1]								
8	9	6.55 ± 0.26	0.73 ± 0.08	0.98 ± 0.14	0.34 ± 0.07	0.30 ± 0.07	0.80 ± 0.09	3.40 ± 0.45
10		4.90 ± 0.33 *	0.68 ± 0.08	0.85 ± 0.17	0.32 ± 0.10	0.32 ± 0.05	0.85 ± 0.08	2.35 ± 0.16 *
20		7.25 ± 0.34 *	0.65 ± 0.14	1.14 ± 0.17 *	0.35 ± 0.05	0.42 ± 0.16	1.36 ± 0.31 *	3.33 ± 0.60 *
Niagara sky blue 6B [2]								
8	8	6.12 ± 0.43	0.90 ± 0.15	1.05 ± 0.18	0.40 ± 0.07	0.33 ± 0.04	0.86 ± 0.12	2.51 ± 0.31
10		5.80 ± 0.41	0.80 ± 0.16	1.15 ± 0.12	0.52 ± 0.05 *	0.73 ± 0.08 *	0.97 ± 0.14	1.66 ± 0.2
20		6.52 ± 0.36 *	0.86 ± 0.38	1.31 ± 0.11 *	0.49 ± 0.13	0.45 ± 0.09 *	1.22 ± 0.2 *	2.20 ± 0.21 *
Congo red [2]								
8	8	6.05 ± 0.29	0.72 ± 0.14	0.97 ± 0.03	0.32 ± 0.03	0.32 ± 0.02	0.72 ± 0.13	3.10 ± 0.43
10		5.70 ± 0.38	0.55 ± 0.13	1.07 ± 0.13	0.34 ± 0.03	0.40 ± 0.03 *	0.84 ± 0.08	2.50 ± 0.63 *
20		6.27 ± 0.80	0.34 ± 0.04 *	0.96 ± 0.14	0.34 ± 0.01	0.39 ± 0.10	1.01 ± 0.15 *	2.63 ± 0.59
Niagara blue 2B [2]								
8	8	6.17 ± 0.44	0.75 ± 0.14	0.88 ± 0.14	0.27 ± 0.04	0.30 ± 0.07	0.82 ± 0.17	3.15 ± 0.6
10		5.70 ± 0.34	0.65 ± 0.12	0.98 ± 0.14	0.26 ± 0.04	0.45 ± 0.08 *	0.84 ± 0.06	2.52 ± 0.26 *
20		5.55 ± 0.17	0.38 ± 0.05 *	0.91 ± 0.28	0.33 ± 0.08	0.46 ± 0.22	1.46 ± 0.12 *	2.01 ± 0.31 *

[1] 14 mg of dye per 100 g maternal body weight.

[2] 20 mg of dye per 100 g maternal body weight.

* Represents a significant change from the preceding value ($P = 0.02$ or less).

sky blue 6B, Congo red, and Niagara blue 2B. Blood samples were taken just before injection, 48 hours after and 12 days after injection. Table 32 presents the findings on the protein content of the serum. In Section X. B.4. the teratogenic effects found in this experiment are presented, but the author did not think there was any connection between these and protein metabolism.

Reuber (1969) gave rats five weekly s.c. injections of trypan blue. Those animals which developed thyroiditis also weighed less than controls or normal-thyroid experimentals; a greater percentage of females developed thyroiditis, but those which did were closer in weight to "normals" than the corresponding males.

Poirier and Pitot (1969) fed rats 0.054% of 2,N,N- or 3',N,N-trimethyl-4-aminoazobenzene for up to 5 weeks. While controls showed a 48% increase in weight, the experimentals gained no weight. Liver weights of controls increased 39%, of 3',N,N fed 24%, and of 2,N,N fed 78%. The 3',N,N animals after 2-3 weeks showed a loss of ability to produce ornithine-δ-transaminase and histidase (after casein hydrolyzate force feeding), and also serine dehydratase. The 2,N,N animals after 3-5 weeks showed a loss of ability to produce tyrosine-α-ketoglutarate-transaminase and serine dehydratase.

Decloitre and Meunier (1970) fed 0.06% of 4-dimethylaminoazobenzene for up to 12 months to male hamsters or the males of two strains of mice, IC and C3H. The hamsters showed no hepatic cellular alterations. The C3H mice showed glycogen and fat deposits in the hepatic cells after six weeks; cellular damage became severe after three months, and continued to increase. The IC mice showed no hepatic alterations until five months. The glycogen and fat deposits appeared at six months along with inflammation.

Hepatic protein-bound dye increased slowly in hamsters to a maximum at six weeks, remained steady for an additional six weeks, then slowly decreased. In the IC mice the maximum (slightly greater than in the hamsters) was reached in two weeks, then gradually decreased (lower than the hamster level at six weeks). The C3H mice reached a maximum at three weeks, about 2 1/2 times greater than the IC mice. This decreased at a rapid linear pace until three months, when it was between the IC's and hamsters. Then it rose slightly at four months, and then dropped quickly to zero at five months (at which point the IC's were slightly above zero, the hamsters considerably above).

Over a five month period the hepatic azoreductase and NADPH-cytochrome c reductase activities were measured. The hamsters showed a fluctuating decrease of 10-42% in azoreductase over this period, and a decrease in the other enzyme over the initial six weeks of 8-30%, and from nine weeks on of 40-50% (both non-time related fluctuations). The C3H mice showed an increase in azoreductase of 10-40% from weeks 2-6, and a decrease of 40% from months 3-5. Their C reductase increased 70-80% in weeks 1-2, then decreased to a value fluctuating around normal from six weeks on. The IC mice showed an increase in azoreductase of 60% after one week, followed by a quick return to normal; the level in months 3-5 seemed a bit below normal, but significance wasn't high. Their c reductase was 50% high at week 2, 30% high at week 3, normal from weeks 4-16, then 50% high at month 5.

Although C3H mice were known to spontaneously develop hepatomas after one year on the control diet used, none of the control mice showed the, apparently, irreversible cell changes occurring in the livers of the dye-fed mice at six months.

Endo et al (1970) fed rats 0.06% of 2,N,N- or 3',N,N-trimethyl-4-

aminoazobenzene. The former did not increase the level of hepatic muscle type aldolase, but the latter did, even after only 15 days of feeding. If the diet were maintained for 60 days, then the increased level of activity was maintained for an additional 300 days.

Poirier and Pitot (1970) fed rats 0.054% of 2,N,N- or 3',N,N-trimethyl-4-aminoazobenzene for up to five weeks in a low-protein, low-riboflavin diet. The animals were then fasted three days, fed a 30% protein diet for 27 hours, and fasted another three days. The livers were then examined for various induced enzyme activities. The 3',N,N dye resulted in zero or diminished responses of glucokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzyme, and citrate cleavage. The 2,N,N dye lowered the 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase, without affecting the others.

Gaunt et al (1971) fed male and female immature rats 50-5000 ppm Orange G (British food grade) for 15 weeks. No effects on food or water consumption or weight gain were seen. Heinz bodies were found in about 10% of the erythrocytes of all animals after two weeks at the 5000 ppm level, and 0.8% after 15 weeks at 500 ppm. Hemoglobin was low at the 5000 ppm level after two weeks in the females, in both sexes after six weeks. Methemoglobin was considerably higher (as percentage of hemoglobin) in both sexes after two weeks at 5000 ppm. Packed cell volume showed a significant decrease (< 10%) in both sexes after six weeks at 5000 ppm. Red blood cells decreased in females at two weeks, males at six weeks at 5000 ppm. Reticulocytes (as percentage of red blood cells) showed a large increase in both sexes after two weeks at 5000 ppm. The serum was analyzed for glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, lactic

dehydrogenase, glucose, urea nitrogen, total protein, and albumin. The only significant change at the $P<0.05$ level was a decrease in glucose in both sexes at six weeks at the 5000 ppm level (but not at 15 weeks). All internal organs were examined but only the spleen showed a weight change - an increase ($P<0.001$) in both sexes after two weeks at 5000 ppm, except that the heart, liver, and adrenals of 15 week females at 5000 ppm showed increases significant at the $P<0.05$ level. On a "relative" base similar results were found except that the significance increased to $P<0.01$ for the heart and liver, decreased below $P<0.05$ for the adrenals, and increased to $P<0.05$ for the female gonads, and ileum ($P<0.01$ at the 500 ppm level) at 15 weeks. In addition the adrenals of both sexes at 5000 ppm after two weeks (only) showed $P<0.05$ increases.

The increase in spleen size was attributed to removal and break-down of the Heinz-bodied erythrocytes. The reticulocytosis was attributed to a compensation for the anemia resultant from the damaged erythrocytes. No explanation for the increased adrenal weight, and only a partial one for the lowered glucose level was offered. The authors concluded that the no-effect level was probably closer to 500 ppm (25 mg/kg body weight/day) than 50 ppm.

Gaunt et al (1971) fed Orange RN (British food grade) - approximately a 6/1 dye mixture - to male and female immature rats at 60-6000 ppm for 15 weeks. Body weight and food consumption were unaffected. Both sexes consumed more water at the 6000 ppm level immediately. Erythrocytes with Heinz bodies appeared at two weeks in both sexes at the 6000 ppm level, in both at the 1200 ppm level at six weeks, and in both at the 600 ppm level at 15 weeks. Hemoglobin dropped in two week females at 6000 ppm, in males at 1200 ppm after six weeks, and at 600 ppm in females at 15 weeks.

Methemoglobin (as percentage of hemoglobin) increased in both sexes at 6000 ppm after two weeks; lower levels were innocuous throughout the experiment. Packed cell volume decreased at 1200 ppm in females after two weeks, and at 6000 ppm in males after six weeks. Red blood cells decreased in both sexes at 6000 ppm after two weeks, in males at 1200 ppm after six weeks. Reticulocytes (as percentage of red blood cells) increased considerably at 6000 ppm in both sexes after two weeks, in both at 1200 ppm after six weeks. Total leukocytes decreased at 1200 ppm (not 6000 ppm) in females after two weeks, at 6000 ppm in males and 1200 ppm (not 6000 ppm) in females after six weeks, in males at 60 and 600 ppm and females at 60 ppm (only) at 15 weeks. The only significant compositional changes in the leukocytes occurred in males at six weeks when the neutrophils showed a large increase at 1200 and 6000 ppm, and the lymphocytes showed a small decrease at the same levels. There were no significant changes in blood chemistry. Examination of the urine showed lower specific gravity in 6000 ppm males at six and 15 weeks, and 1200 and 6000 ppm females at 15 weeks (all 0-6 hour specimens), and in 6000 ppm females at 15 weeks (16-20 hour specimen). An increase in urine volume was noted in 6000 ppm males at 15 weeks (both time periods), and in 1200 ppm females at 15 weeks (0-6 hours). Organ weight increases occurred in the spleen - 6000 ppm females at two weeks, 1200 and 6000 ppm both sexes at six and 15 weeks, and in the liver - 6000 ppm females at 15 weeks. Relative organ weights showed similar increases for the spleen except for addition of the male at two weeks/6000 ppm and deletion of the male at 15 weeks/1200 ppm; other organs showing relative changes were: brain - decrease in 15 week males at 600 and 1200 ppm; liver - increase in two and 15 week females at 6000 ppm; thyroid - decrease in two week males at 1200 ppm.

The authors could not confirm any relationship between the increased water consumption at 6000 ppm and the more dilute urine at that level. The changes in white blood cells and decreased relative brain weights were not considered related to the dye feeding. The no-effect level of the dye was considered to be 60 ppm (3 mg/kg body weight/day), with 600 ppm being reasonably safe.

Yen et al (1971) gave i.p. injections of trypan blue at 50, 100, and 350 mg/kg to rabbits. The lower two doses had no effect on dentin formation, while the high dose completely inhibited it for eight days.

Tschopp et al (1971) gave i.v. doses of Congo Red, 20-80 mg/kg, to cats and rabbits. The two higher doses caused an immediate drop in blood platelets (80-90%), there being no recovery for two hours after the highest dose, and only partial recovery after the middle dose. The middle dose had a similar effect on leukocyte count, but recovery was complete in about 20 minutes, followed by attainment of a long lasting plateau of excess leukocytes (3 times normal) in 30 minutes. The immediate effect on leukocytes of the high dose was not clear, but a gradual increase to well above normal followed. In contrast, in vitro incubation of citrated blood with Congo Red equivalent to 50 and 100 mg/kg did not affect platelet or leukocyte count. Remaining platelets showed extensive swelling and pseudopod formation. Within two hours the lungs showed alveolar edema and capillary obstruction, with larger vessels containing large numbers of leukocytes and platelet aggregates.

Popa et al (1971) reported that 0.1 mM toluidine blue totally inhibited the synthesis of DNA-dependent, highly polymerized RNA in KB cell culture.

Motoc et al (1971) gave rats 25 mg, orally, of azobisisobutyronitrile

twice a week for three months. Some lesions were found in the stomach, liver, and kidneys which seemed to be reversible. These correlated in intensity with decreases in serum glycoprotein and albumin, and increases in leucine aminopeptidase, glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, glucose-6-phosphate dehydrogenase, aldolase, and β-glycuronidase.

Iga et al (1971) gave adult male rats 30 μmole i.v. doses of the dyes amaranth and new coccine and studied the simultaneous excretion in bile and elimination from serum. In four hours 80% of the amaranth had appeared in the bile (mostly in the initial hour and a half), but only 10% of the new coccine. The latter was eliminated from the serum at a slower rate than amaranth: serum half lives were 20 1/2 min. and 5.8 min., respectively. The authors examined the ability of the plasma protein to bind with the dyes, and decided the difference could account for only a small part of the biliary excretion spread.

Holland and Spain (1971) fed immature male rats 0.06% of 3',N,N-trimethyl-4-aminoazobenzene for 36 and 66 day periods. Examination of the shorter period feces for bile acids showed normal amounts of lithocholic, deoxycholic, chenodeoxycholic, somewhat lesser amounts of 12-keto-lithocholic and hyodeoxycholic, and considerably less cholic. The urine collected from the controls showed the bile acids hyodeoxycholic (day 15) and lithocholic (day66); the dye-fed animals' urine also showed these two, but considerably more cholic, hyodeoxycholic and ursodeoxycholic.

Hepatic bile duct oval cells increased from 1% in controls to 41% at day 46, dropping to 36 by day 53 and staying there. Urinary bile acids peaked at 26 and (lower) 53 days.

Gafford et al (1971) fed male rats 1-10% of azobisformamide in a low-iodine diet for up to four weeks, or gave i.p. doses daily for one week of

0.2-20 mg/kg body weight. One day prior to sacrifice each rat was given radioiodine, i.p. With oral dosing all levels led to reduced iodine uptake (thyroidal), especially at 5-10% over 10-28 days. Total body weight decreased about 10% ($p<0.02$) after one week at 10%. Relative thyroid weight increased over 20% ($p<0.1$) after one week at 1%, 40% ($p<0.05$) after one week at 10%, and decreased <20% ($p<0.001$) after 10 days at 5%. Serum protein bound iodine was lower in the azo fed animals.

In the i.p. dosage the maximum level produced lower iodine uptake (but only at the $p<0.5$, 0.9 level). Relative thyroid weight was unaffected. Total body weight decreased 10% ($p<0.1$) at the maximum level in one run, not at all in another. The authors concluded that permissible levels of the azo compound in flour are of no concern in thyroid activity.

Gaunt et al (1971) gave immature male and female rats 100-10000 ppm of Yellow 2G (British food grade) for 13 weeks. No effect on body weight, food or water consumption was noted. Hematological results were neutral, likewise serum analyses. Urine examination was without notable findings. Absolute weights of internal organs showed an increase in kidneys in two week females at 10000 ppm, a decrease in the ileum of six week males at 1000 and 10000 ppm, an increase in the adrenals of six week females at 1000 ppm, and a decrease in terminal body weight of 13 week males at 10000 ppm - 24 hour fasting was procedure prior to sacrifice and autopsy. Relative weight changes showed an increase in kidneys of 13 week males at 1000 and 10000 ppm, an increase in the caecum of six and 13 week males at 10000 ppm and 13 week females at 10000 ppm, and an increase in the gonads of 13 week males at 1000 ppm.

The increase in relative kidney weight was judged to be non-dye related. The increase in caecal weight has doubtful human significance.

The increase in testes weights was also deemed of dubious significance. The authors recommended a no-effect level of 1000 ppm (80 mg/kg body weight/day), or about 2000 times expected maximum intake.

Gaunt et al (1972) gave immature male and female rats 1000-10000 ppm of Black PN for up to two years. Mortality was considered equivalent to the controls. Body weight of controls and dye-fed animals was equivalent. Hemoglobin was lower in 82 week females at 10000 ppm, and 105 week males at all levels. Packed cell volume was lower in the 1000 and 5000 ppm groups of these males. Red blood cells were higher in 82 week females at 5000 ppm. Total leukocytes were lower in 105 week females at 10000 ppm. Serum and urinary biochemical analyses were normal at 52 and 104 weeks. Absolute organ weights were all normal at 104 weeks. Relative organ weights were normal except for male liver which was heavier at all levels. Histological examination of lungs, kidneys, pancreas, liver, and testes showed no abnormal occurrences in dye-fed animals. Incidence of tumors in the mammary, pancreas, thyroid, and adrenal glands was normal, likewise that in the ovary and subcutaneous tissue. The authors recommended a no-effect level of 10000 ppm (500 mg/kg body weight/day), about 2000 times maximum expected intake.

Lin et al (1972) gave i.p. injections to rats of 0.3 mmoles/kg body weight of 4-amino-, 4-methylamino-, and 4-dimethylaminoazobenzene. Analysis of the blood for methemoglobin (as percentage of hemoglobin) showed a rapid rise to 70, followed by a linear drop to 10 at 7 hours for 4-amino-, a rapid rise to 50, followed by a plateaued drop to 10 at 13 hours for 4-methylamino-, and a slow, irregular rise to 25 at 7 hours, followed by a linear drop to 10 at 13 hours for the 4-dimethylamino- compound. An in vitro study failed to generate methemoglobin, implying metabolites were

responsible. Examination of possible metabolites implicated N-hydroxylation as a highly likely preliminary step.

Singh and Khanna (1972) gave rats a single injection into one testis of 0.1 ml of a 1% solution/100 g body weight of C.I. Acid yellow 36. Examination of the testis was conducted at 0-16 hours, 1-15 days. At four hours there were signs of edema and inflammation. At eight hours both had increased in intensity and were accompanied by seminiferous tubule degeneration, Leydig cell degeneration, and engorgement of blood vessels. At 16 hours the edema had decreased, but now the interstitium had begun to degenerate where associated with the tubules also degenerating. After one day no increase in magnitude of changes had occurred, but in half of the specimens the seminiferous and interstitial elements had totally degenerated. After two days massive degenerative changes showed in the gametogenic and endocrine elements. Spermatozoa had decomposed here and there. After seven days the tubules were totally necrosed along with the interstitial elements. After 15 days the interstitium shows signs of regeneration, but the tubules did not.

Olsen and Hansen (1973) fed male and female pigs 10-160 mg/kg body weight/day doses of Orange RN for three months. At the highest dose there was severe hepatic interstitial fibrosis and multiple nodular hyperplasias of the parenchyma. All dosages caused proliferation of hepatic bile ductule epithelium cells along the triads and the interlobular septa, sometimes intrahepatically also.

IX. ENVIRONMENTAL EFFECTS

A. Persistence and/or Degradation

Mecke, Jr. and Schmahl (1957) reported a study which might have some bearing on environmental longevity. They incubated a variety of azobenzene derivatives with fresh baker's yeast and determined the extent of decolorization - possibly synonymous with azo cleavage. Azobenzene did not decolorize, nor did the 2-, 3-, or 4-methyl-, or the 4, 4'-bis(dimethylamino)- derivative. Also not decolorizing were phenylazo-2-(N-methyl)naphthylamine, o-tolylazo-2-naphthylamine, and anisidineazo-2-naphthol. The 4-sulfo- derivative only decolorized to the extent of 4%. In the 10-35% range were 2-methyl-4-hydroxy-, 4-dimethylamino-4'-methyl-, 2-hydroxy-4-dimethylamino-, phenylazo-2-naphthylamine, o-tolylazo-2-naphthol, and phenylazo-2-naphthol. In the 45-75% range were 2-aminoazotoluene, 2-hydroxy-, 4-hydroxy-, 3-methyl-4-hydroxy-, 2,4-dihydroxy-, sodium 4-dimethylamino-4'-sulfo-, sodium 2,4-dihydroxy-2',4'-disulfo-, and naphthylazodimethylaniline. In the 80-99% range were 4-amino-, 4-dimethylamino-, 4'-carboxy-4-dimethylamino-, and 2-carboxy-4-dimethylamino-. Solubility in water apparently was not a factor.

Walker et al (1971) removed azoreductases from various rat gut bacteria and compared their ability to reduce Red 2G. The most proficient came from Streptococcus faecalis. The following activities relative to S. faecalis were reported: S. faecalis var. zymogenes-0.95, S. faecum -0.79, E. coli type 1 -0.62, Proteus vulgaris -0.58, P. mirabilis -0.51, P. morganii -0.49, and Staphylococcus aureus -0.02.

B. Environmental Transport

No specific information was found. The azo dyes and foaming agents investigated are not notably volatile, so their ability to move once released into the environment is dependent upon water solubility (and specific gravity for the non-water solubles).

C. Bioaccumulation

No specific information was found. Intestinal bacteria seem able to destroy the azo bond in many compounds. Some metabolism tests indicated "storage" of various azo dyes in skin and fur on prolonged forced feeding, but to no obvious detriment of the animal.

X. TOXICITY

A. Human-Occupational experience, Other

1. acute, subacute

Hoffman and Guz (1961) on three occasions subjected themselves to injections of Coomassie blue. On the first, seven doses over five hours totalled 150 mg in the first three hours and 300 mg in the last two. On the second, at nine-minute intervals doses of 40, 48, 78, 168, 188, and 276 mg were given; maximum blood level reached 99.5 mg/l. On the third, at nine-minute intervals doses of 40, 36, 72, 161, 186, 194, and 235 mg were given; maximum blood level was 138.5 mg/l. Each time a 2-3 hour period of no effects was followed by 10-15 minutes of ill-feeling, and then fever up to 40°C(104°F), rigors, hyperesthesia of skin and muscle, nausea, vomiting (blue color), and, once, diarrhea (blue color). All symptoms subsided after 5-6 hours. Minor periodic sweating/fever recurred for an additional 2-3 days. Subsequently, as much as 54 mg in eight hours or 50 mg in 4-5 hours

produced no ill effects in the authors or other subjects. Accidental perivenous administration produced severe pain after 2-3 hours.

Hörstensmeyer (1964) reported a fatal reaction after i.v. injection of Congo Red in man (mentioned in Tschopp et al [1971]).

Cohen and Bovasso, Jr. (1971) reported on the accidental ingestion by a 13-month old child of 2500-3000 mg of phenazopyridine·HCl (Pyridium). Apart from cyanosis of the lips, the only outward symptom was lethargy. On admission to a hospital within about four hours, the blood methemoglobin was found to be at least 25%. After another 14 hours this had dropped to 14.6%; normal methemoglobin is 1.7%. Normal methylene blue treatment for methemoglobinemia proved inadequate, but transfusion corrected the situation.

2. chronic

No reports were found dealing with chronic toxicity of azo compounds in humans.

3. sensitization

Meara and Martin-Scott (1953) reported three separate cases of women developing skin sensitization to aminoazotoluene, apparently from contact with ball point pen ink containing it.

Foussereau et al (1971) discussed the rather common skin allergy to Disperse Yellow 3, apparently known for many years. They showed that any impurities present in commercial material were no more allergenic than the dye itself.

4. teratogenicity

5. carcinogenicity

6. mutagenicity

7. behavioral effects

No reports were found disclosing these properties of azo compounds in humans.

B. Birds and Mammals

1. acute, subacute

Zsolnai (1963) reported the following LD-0 and LD-100 doses in rats after i.p. administration, in mg/kg: 1. aryl-azo-malonitriles--phenyl, 5, 15; 2-tolyl, 5, 15; 3-tolyl, 5, 20; 4-tolyl, 5, 30; 2-chlorophenyl, 5, 15; 3-chlorophenyl, 5, 10; 4-chlorophenyl, 5, 10; 4-bromophenyl, 5, 10; 4-iodophenyl, 5, 10; 4-ethoxyphenyl, 40, 70; 2-methyl-4-bromophenyl, 5, 15; 2-methyl-4-iodophenyl, 5, 20; 3-methyl-4-bromophenyl, 5,15; 4-methyl-2-bromophenyl, 20, 30; 2-bromo-4-ethoxyphenyl, 40, 60; 2,5-dichlorophenyl, 5, 15; 3,5-dibromophenyl, 5, 15; 2-chloro-4-bromophenyl, 10, 20; 3-chloro-4-bromophenyl, 10, 20; 2-methyl-4,6-dibromophenyl, 10, 20; 2-chloro-4,6-dibromophenyl, 20, 60; 3-chloro-4,6-dibromophenyl, 5, 20; 4-chloro-2,6-dibromophenyl, 20, 40; 2,4,6-tribromophenyl, 30, 60; 2-nitrophenyl, 5, 10; 3-nitrophenyl, 20, 30; 4-nitrophenyl, 10, 20; 2-methyl-4-nitrophenyl, 5, 10; 3-methyl-4-nitrophenyl, 10, 20; 4-methyl-2-nitrophenyl, 10, 20; 2-nitro-4-ethoxyphenyl, 10, 20; 2-chloro-4-nitrophenyl, 5, 10; 3-chloro-4-nitrophenyl, 10, 20; 4-chloro-2-nitrophenyl, 5, 10; 4-acetylaminophenyl, 100, 400; 2-carboxyphenyl, 100, 150; 4-carboxyphenyl, 100, 300; 4-carboethoxyphenyl, 20, 30; 3-hydroxy-4-carboxyphenyl, 200, 500; 4-sulfophenyl, 300, 800; 4-sulfonamidophenyl, 200, 500; 4-N-(4',6'-dimethyl-2'-pyrimidyl)sulfonamidophenyl, 400, 600; 1-naphthyl, 10, 20; 4-bromo-1-naphthyl, 20, 50; 4-phenylazophenyl, 10, 20;

diphenylene-4,4'-bis-(azomalonitrile), 500, 800; 3,3'-dimethyldiphenylene-4,4'-bis-(azomalonitrile), 30, 50; 2. aryl-azo-cyanoacetic acid esters--phenyl/methyl ester, 600, > 800; 4-tolyl/methyl ester, > 800, --; 4-chlorophenyl/methyl ester, 200, 600; phenyl/ethyl ester, 400, 800; 4-tolyl/ethyl ester, > 800, --; 4-chlorophenyl/ethyl ester, 200, 600; 3. aryl-azo-cyanacetamide and its N-substituted derivatives--phenyl, > 800, --; 4-tolyl, > 800, --; 4-chlorophenyl, > 800, --; phenyl/phenyl, > 800, --; 4-tolyl/phenyl, > 800, --; 4-chlorophenyl/phenyl, > 800, --; phenyl/4'-chlorophenyl, > 800, --; 4-tolyl/4'-chlorophenyl, > 800, --; 4-chlorophenyl/4'-chlorophenyl, > 800, --; phenyl/amino, > 800, --; 4-tolyl/amino, > 800, --; 4-chlorophenyl/amino, > 800, --; 4. other--phenylazoacetylacetone, 600, 800; 3-tolylazoacetylacetone, 400, 800; 4-tolylazoacetylacetone, 100, 200; 3-chlorophenylazoacetylacetone, > 800, --; 4-chlorophenylazoacetylacetone, > 800, --; phenylazoacetic ester, 100, 300; 3-tolylazoacetic ester, 400, 600; 4-tolylazoacetic ester, 400, 600; 3-chlorophenylazoacetic ester, 400, 600; 4-chlorophenylazoacetic ester, 200, 300; phenylazodiethyl malonate, > 800, --; 4-tolylazodiethyl malonate, > 800, --; 4-chlorophenylazodiethyl malonate, > 800, --.

Niculescu-Duvaz et al (1966) reported that the LD-50 for 4,4'-dihydroxyazobenzene and for the 4,4'-bis[$-O_2CN(CH_2CH_2Cl)_2$] azobenzene was 300-500 mg/kg.

Chadwick et al (1966) reported LD-50's in mice after i.p. injection, in mg/kg, for: 2,4,6-triamino-5-(4-carbethoxyphenylazo)-6-N-(2-hydroxy-3-anilino)propylpyrimidine, > 1600; the same, except chloro in place of hydroxy, 300; the same, except no 2-substituent on the propyl, 1100.

Sato et al (1966) reported that in immature female rats repeated injections of N-hydroxy-4-aminoazobenzene in a dose larger than 3.6 mg/100 g proved very toxic, death resulting from methomoglobinemia.

Grasso et al (1968) studied the toxicity of Brown FK in laboratory animals; chronic results are given in the next section. Results of the acute study are in Table 33, on rats and mice, 14 and 7 day observation periods, respectively.

Table 33. *Acute toxicity of Brown FK in rats and mice by oral intubation and intraperitoneal injection*

Species	Route	Single dose (g/kg)	Deaths/group of 5 animals	
			Male	Female
Mouse	Oral	2·0	0	0
	Intraperitoneal	1·0	0	0
		1·5	2	1
		2·0	5	5
Rat	Oral	2·0	0	0
		4·0	1	0
		8·0	1	1
	Intraperitoneal	0·75	0	–
		1·15	4	–
		1·69	5	–

Autopsy failed to reveal the cause of death.

Gaunt et al (1969) reported oral LD-50's for Ponceau 4R in rats and mice of >8 g/kg, and i.p. LD-50's of 1.6-1.9 g/kg in mice and 2.6 g/kg in female rats; male rats had about the same i.p. LD-50 as females, after 48 hours, but after seven days the male value dropped to 0.6 g/kg (all values taken from a 1967 publication by Gaunt et al). On autopsy, renal tubular necrosis was found. In 1957 a German report

quoted LD-50 values for rats of 2 g/kg i.p. and 1 g/kg i.v.

Gaunt et al (1969 pp. 557-563) reported oral LD-50's for Black PN to be greater than 5 g/kg in mice (1957 German report) and also in rats (1967 Gaunt et al). In the earlier Gaunt report i.p. LD-50's of 0.5-1.0 g/kg for mice and 0.9-1.2 g/kg for rats were also disclosed.

Caldwell et al (1971) reported minimum lethal oral doses in mice for some azo derivatives of the 3-tropanyl (R) ester of 2,3-diphenylacrylic acid (in mg/kg): 256 for $[C_6H_5CH = C(CO_2R)C_6H_4\text{-}m\text{-}N\overline{+}_2$, 768 for $[C_6H_5CH = C(CO_2R)C_6H_4\text{-}p\text{-}N\overline{+}_2$, 768 for $C_6H_5CH = C(CO_2R)C_6H_4\text{-}m\text{-}N = N\text{-}C_6H_5$, >1024 for $C_6H_5CH = C(CO_2R)C_6H_4\text{-}p\text{-}N{=}N\text{-}C_6H_5$, and >1024 for $C_6H_5CH = C(CO_2R)C_6H_3(\text{-}p\text{-}OH)\text{-}m\text{-}N = N\text{-}C_6H_5$.

The Toxic Substances List-1973 Edition provided the following compilation of acute toxicities, including the year of publication of their source. LDLo, TCLo, and TDLo are their abbreviations for lowest published lethal dose, toxic air concentration, and toxic dose, respectively. The parenthetical chronologies refer to observation periods.

Acid Red 26 LD-50, mice, i.p., 2 g/kg, 1966

4-Aminoazobenzene (AAB) LDLo, mice, i.p., 200 mg/kg
TDLo, frogs, i. renal, 110 mg/kg, 1964

4-Amino-N,N-bis(2-chloroethyl)-2'-carboxy-2-methylazobenzene
LD-50, rats, i.p., 20.2 mg/kg, 1964

4-Amino-N,N-dimethylazobenzene (DAB)
LD-50, mice, i.p., 500 mg/kg, 1962
TDLo (1 week), mice, oral, 5 mg/kg, 1958
TDLo (40 days), rats, oral, 800 mg/kg, 1956

4-Amino-2'3-dimethylazobenzene
TDLo, mice, s.c., 330 mg/kg, 1965

4-Amino-N,N-dimethyl-4'-fluoroazobenzene
TDLo(12 weeks), rats, oral, 3.2 g/kg, 1953

4-Amino-3',5'-dimethyl-4'-hydroxyazobenzene
LD-50, rats, i.p., 350 mg/kg, 1963
TDLo, rats, i.d., 100 mg/kg, 1963

4-Amino-4'-hydroxyazobenzene
LD-50, rats, oral, 1.95 g/kg, 1963
LD-50, rats, i.p., 300 mg/kg, 1963

4-Amino-4'-hydroxy-2,3',5'-trimethylazobenzene
LD-50, rats, i.p., 142 mg/kg, 1963
LDLo, rats, oral, 600 mg/kg, 1963
TDLo, rats, i.d., 100 mg/kg, 1963

4-Amino-2',N,N-trimethylazobenzene
TDLo (6 weeks), rats, oral, 1.5 g/kg, 1969

Azobenzene LD-50, rats, oral, 1 g/kg, 1966

Azobisisobutyramide·HCl
LDLo, rats, oral, 400 mg/kg, 1971

Azobisisobutyronitrile (AIBN)
LD-50, rats, oral, 700 mg/kg
LDLo, mice, i.p., 25 mg/kg

Azoethane TCLo, rats, inhal., 4800 ppm/hr, 1968

1,1'-Azonaphthalene TDLo, mice, s.c., 200 mg/kg, 1940

1,2'-Azonaphthalene TDLo, mice, s.c., 200 mg/kg, 1940

Congo Red LD-50, rats, i.v., 190 mg/kg

Food Brown 3 LD-50, rats, i.p., 375 mg/kg, 1966

Food Red 3 LD-50, rats, i.p., 1.1 g/kg, 1967

Food Yellow 3 LD-50, mice, i.p., 4.6 g/kg, 1967
(FD&C Yellow 6) LD-50, rats, i.p., 3.8 g/kg, 1967

Solvent Red 24 TDLo, rats, s.c., 8.32 g/kg, 1958

Solvent Red 80 TDLo, mice, i.p., 80 mg/kg, 1968

Trypan Blue LD-50, mice, i.v., 267 mg/kg, 1970
LDLo, rats, i.v., 300 mg/kg

2. chronic

Metcalf (1962) gave adult rats three s.c. doses of 20 mg of Evans Blue and Trypan Blue on alternate days (separate experiments for each dye). This treatment resulted in death of the rats in three weeks. The i.v. LDLo for Trypan Blue in rats, above, corresponds to 60 mg per 200 g rat, an interesting, though not directly comparable, comparison.

Davis and Fitzhugh (1963) fed male and female rats 0.01-1.0% of D&C Red No. 10 for two years. There was no effect on growth. There was a definite increase in longevity at the 1% level. Survivors' splenic weight (relative) increased 2-3 times in both sexes at the 0.25 and 1% levels. Slight to moderate bone marrow hyperplasia was noted in the 0.25% females and both sexes at 1%. Frequency of occurrence of the wide variety of tumors found was not significantly different from that in the controls.

Oser et al (1965) treated for two years male and female dogs and rats with a diet composed mainly of bread (overall nutritionally balanced for each animal) which had been made with 100 ppm of azobisformamide. This level was ten times the proposed use level, about twice the maximally permitted level, and was all that could be incorporated without interfering with the baking process. There were no adverse effects on the original animals, or on three subsequent generations of rats.

Chadwick et al (1966), in an anti-tumor study, gave rats i.p. doses on five successive days of 2,4,6-triamino-5-(4-carbethoxyphenylazo)-6-N-(3-anilino)propylpyrimidine, and its derivatives with

chloro or hydroxy substitution on the 2-propyl position. They found approximate LD-50 values of 550 mg/kg for a 400 mg/kg/day dose for the parent compound, 140 mg/kg for a 100 and 50 mg/kg/day (not clear in the paper) dose for the 2-chloro, and >400 mg/kg for a 400 mg/kg/day dose for the 2-hydroxy.

Ikeda (1966) fed rats 0.2-5.0% of Ponceau MX for up to 15 months. Mortality was the same as the control group. At 1 and 5% growth was noticeably retarded even though food consumption was normal; water consumption in these two groups increased after the eighth month. All levels produced heavier than normal livers and thyroids from at least the third month. The kidneys were heavier at the 1 and 5% levels. Liver cell adenomas were seen in dead animals as early as 10 months, the incidence increasing with amount of dye fed. Obvious renal tubular degeneration had occurred by three months at even the 0.2% level. By the 15th month all groups (including the control) showed glomerulonephritic and nephrotic changes, also interstitial cell infiltration. Incidence and severity were greater in the dye-fed animals than the controls.

In an in-progress study on feeding the same range of this dye to mice for up to 12 months, liver tumors were found at the 0.2% level.

Grasso et al (1968) did a chronic toxicity study on various laboratory animals of Brown FK and some of its isolated azo components. Rats and mice were given in their diet for up to 43 days 0.1-2.0 g/kg of Brown FK, or by i.p. injection 43 doses of 0.1-1.0 g/kg. Guinea pigs, hamsters, and rabbits were given stomach tube doses

of 1 g/kg Brown FK for up to 14 days. Rats received up to 16 daily doses by stomach tube of the FK components 2,4-diamino-5-(p-sulfophenylazo)toluene (I), 1,3-diamino-4-(p-sulfophenylazo)benzene (II), 2,4-diamino-3,5-bis(p-sulfophenylazo)toluene (IV), or 1,3-diamino-2,4,6-tris(p-sulfophenylazo)benzene, a 40/60 mixture of I/IV, or a mixture of II, VI, 1,3-diamino-4,6-bis(p-sulfophenylazo)benzene (III), and 1,3-diamino-2,4-bis(p-sulfophenylazo)benzene (V); the dose was 0.5 g/kg. Similarly, 1 g/kg doses were given of 50/50 I/II, 40/60 I/IV, and I/II/III/V/VI (50% I).

The 1 g/kg i.p. doses of FK had no effect on mice. The 2 g/kg oral doses (six) had no effect on mice. After 28 oral 1 g/kg doses, one out of twelve mice showed heart and skeletal muscle changes.

Both sexes of two strains of rats died after receiving 3-8 doses (stomach tube) of 1 g/kg of FK. The observed symptoms were, sequentially, growth retardation, heavy weight loss, lethargy, piloerection, hypothermia, difficult breathing, death. Sacrifice of the animals just after weight loss was noticed revealed greyish-white areas in the ventricular myocardium, conjested liver and lungs. Lesions were found in the heart and skeletal muscles.

Of the rats given 1.5 g/kg of FK, one-third developed centrilobular hepatic necrosis and severe fatty change.

The following tables condense the muscle damage information obtained from stomach tube dosing.

Table 34. *Incidence of striated-muscle damage induced by Brown FK in relation to daily dose administered by stomach tube to rats*

Daily dose (g/kg)	No. of doses		No. of rats examined	Percentage of rats affected			
	Range	Mean		Heart	Tongue	Skeletal muscle	Diaphragm
0·0	2–43	23	60	0	0	0	0
0·1	5–43	26	10	10	10	0	10
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0	10	6
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	33	33	22
[illegible]	[illegible]	[illegible]	51	72	67	52	65
[illegible]	[illegible]	7	10	10	—	—	—
1·5	[illegible]	6	10	90	—	—	—
2·0	[illegible]	4	10	90	—	—	—

Only animals that survived doses above 1 g/kg.

Table 35. *Incidence of striated-muscle damage induced by components of Brown FK administered by stomach tube to rats*

Material administered	Daily dose (g/kg)	No. of doses*	No. of rats examined	Percentage of rats affected			
				Heart	Tongue	Skeletal muscle	Diaphragm
Saline	—	6–16	20	0	0	0	0
Brown FK	1·0	2–16	16	69	94	75	81
Compound I	0·5	2–6	9	100	89	22	44
Compound II	0·5	2–6	10	30	50	30	40
Compounds I+III†	1·0	2–3	12	75	100	100	100
Compounds I+IV‡	0·5	6	10	0	0	0	0
Compounds I+IV‡	1·0	3–16	10	10	30	0	30
Compound IV	0·5	16	10	0	0	0	0
Compound VI	0·5	7–12	6	0	0	0	0
Compounds II+III+V+VI	0·5	6	11	0	0	0	0
Compounds I+II+III+V+VI†	1·0	5–6	10	50	70	40	50

*Results represent findings from two studies in which rats were dosed for up to 6 or 16 days respectively.
†Mixture containing 50% I.
‡Mixture containing 40% I.

Table 36. *Incidence of striated-muscle damage induced in various species by Brown FK (1 g/kg/day) administered by stomach tube*

Species	No. of doses	No. of animals examined	Percentage of animals affected			
			Heart	Tongue	Skeletal muscle	Diaphragm
Rat	1–6	12	85	72	65	62
Guinea-pig	3–11	12	33	59	59	75
Rabbit	3–9	11	91	64	55	82
Mouse	6	12	0	0	0	0
Hamster	14	12	0	0	0	0

For each species, groups of similar size were dosed with water and no lesions were seen.

The i.p. dosing was more toxic. Although the 0.1 g/kg regimen was well tolerated, the 1 g/kg level was usually fatal after 10 shots. Only three 1 g/kg shots sufficed to greatly reduce splenic weight, and increase relative renal weight. The renal tubules were dilated, vacuolated, and pigmented with FK (not pigmented at all after oral dosing). The i.p. dosing did not produce the muscle damage seen from oral dosing, indicating metabolites were probably responsible. It was suggested that, since some animals were unaffected by even the highest doses, there was intraspecies variation in the gut microflora apparently responsible for the metabolizing of the azo linkages.

Grasso and Golberg (1968) reviewed the rather extensive literature on metabolism and toxicity of Brown FK, using some lessons learned to comment on the nature of the testing procedures for food dye toxicity.

Gaunt et al (1969) fed male and female pigs Ponceau 4R at 100-900 mg/kg body weight/day for 90 days. There was no effect on weight gain. Hematological findings were normal at 300 mg/kg/day. At 900 mg/kg/day males showed reduced hemoglobin and red blood cells at week six but not at week 13. Urine and serum chemical analyses were normal. Relative internal organ weights were also considered normal. Autopsy and histopathology on sacrifice of the animals revealed no abnormalities.

Grasso et al (1969) fed male and female rats 0.125-1.0% of Ponceau MX (also known as 2R) for up to two years. Fatalities became statistically significant at $P < 0.05$ by the 88th week in both sexes at the 0.5% level. However, by the 80th week all levels in males and

0.25% up in females showed somewhat higher mortality. The 0.125% level in females never did produce unusual mortality. Body weight increase showed a slowdown in females at 1% by the 3rd week, at 0.5% by the 25th week, at 0.25% by the 51st week, and at 0.125% by the 65th week; there wasn't any noticeable reduction in the weight of females from a peak weight. In males initial slowdown began later, by week 39 at the 1% level, by week 65 at levels 0.25 and 0.50%, and by week 91 at 0.125%; males showed sharp, continuing drops in weight between weeks 65 and 77 at levels 0.25% up, and between weeks 65 and 91 at 0.125%. Examination of food eaten at consecutive three-month intervals showed decreases by females only over the three intervals covering weeks 54-93 at the 0.5% level; males showed decreases over the 94-99 week period at 0.125%, 80-99 weeks at 0.25 and 0.5%, and 66-99 weeks at 1.0%. The animals didn't care for the taste of the dye even at the 0.125% level, but the palatability showed a very high decrease going from 0.5 to 1.0%.

Hematology revealed decreases in hemoglobin in males by week 14 at the 0.5 and 1.0% levels and in females at the 1% level; by week 29 females were showing decreases at the 0.25% level and were also showing decreases in hematocrit at that level and up. By week 61 males were showing lower hemoglobin at 0.125%, lower hematocrit at 0.25%, and lower red blood cells at 0.25%. At week 79 only hemoglobin was low at the highest level in both sexes, except that males showed a higher total leukocyte count at 0.125%. At week 104 hemoglobin was low in both sexes at 0.25%, also hematocrit; in males the red blood cells were also low at that level, also total leukocyte count. Chemical analysis of the blood was normal after two years. Urine analysis showed higher ascorbic acid after one year at 0.25% up, otherwise being normal chemically and physically at

one and two years. Relative internal organ weights after two years showed increases in the brain of both sexes at 0.5% (no 1% values for any organs were determined because of too few survivors), heart of males at all levels, liver of both sexes at all levels except 0.125 in females, kidneys of both sexes at all levels, adrenals of both sexes at 0.5%, and pituitary of males at 0.125 and 0.5%. Incidence and severity of pathological hepatic changes were directly related to dose level, but there was nothing to suggest carcinogenicity.

Gaunt et al (1969) fed male and female pigs Black PN at 100-900 mg/kg/day for 90 days. There was no effect on body weight. Hematology was normal. Relative internal organ weights were normal. There seemed to be evidence of ileal irritation at 300 and 900 mg/kg/d, possibly resultant from the feeding of the daily dose in a highly concentrated form.

Gafford et al (1971) gave male rats daily i.p. doses of 0.2-20.0 mg/100 g body weight of azobisformamide. The high dose caused 62% mortality in one week, starting on the third day. The deaths were preceeded by 1-2 days of bloody urine.

Galea et al (1972) gave rats 30 mg/d of Amaranth for up to 545 days, as 0.12% of their diet. Between 60 and 180 days weight began to lag behind the controls, but the absolute difference didn't increase between 180 and 360 days. Apparently there was considerable mortality in the dosed animals, but inconsistencies in the paper obscured the results. Hepatic vitamin A was only half that of controls at 60 days, 20% at 180 days, and 0-10% at the end of the experiment.

Shtenberg and Gavrilenko (1972) fed male and pregnant female rats a daily dose of 1.5 mg/kg or 15 mg/kg Amaranth for 12 months.

At the end of the test period both levels had about the same ability to reduce spermatosoidal resistance, depress the estral cycle, and heighten the gonadotropic function of the hypophysis. Postimplant mortality was far higher, 3-5 fold. The number of live births per rat dropped 30%. The fetal and placental weights were slightly lower at 15 mg/kg. The 1.5 mg/kg level was the maximum recommended at the 8th FAO/WHO session on food additives.

Collins et al (1972) gave pregnant rats 7.5-200 mg/kg/day of Amaranth during days 0-19 of gestation. They found no change in the number of corpora lutea, nor any adverse effect on implantation. Fetal mortality increased notably at a dosage over 15 mg/kg. Weight of live young was not affected. Percentage of resorptions increased with dose. Skeletal and soft-tissue abnormalities were not dose related.

The Toxic Substances List-1973 Edition provided the following compilation of chronic toxicities; see the last entry under Section X.B.1. for elaboration of terms.

Acid Red 26 TDLo, rats, oral, 62 g/kg/65 weeks, 1966

Acid Red 148 mice, s.c., 80 mg/kg/4 weeks, 1950
(intermittent dosage)

4-Amino-N,N-dimethylazobenzene (DAB)
TDLo, hamsters, oral, 9.6 g/kg/42 weeks, 1961
(intermittent dosage)

4-Amino-2,4'-dimethylazobenzene
TDLo, mice, oral, 28 g/kg/595 days, 1949

4-Amino-2,3'-dimethylazobenzene
TDLo, mice, oral, 28 g/kg/595 days, 1949

4-Amino-2',3-dimethylazobenzene
TDLo, hamsters, oral, 25 g/kg/49 weeks, 1961
TDLo, mice, oral, 2.8 g/kg/70 days, 1955

4-Amino-3,4'-dimethylazobenzene
TDLo, mice, oral, 28 g/kg/595 days, 1949

4-Amino-3',5'-dimethyl-4'-hydroxyazobenzene
TDLo, rats, oral, 1000 ppm/2 years, 1963

4-Amino-4'-hydroxyazobenzene
TDLo, rats, oral, 700 ppm/2 years, 1963

Food Red 1 TDLo, rats, oral, 1.2 g/kg/day, 1953

Food Red 6 TDLo, rats, oral, 182 g/kg/2 years, 1963

Food Yellow 3 (FD&C Yellow 6) TDLo, mice, oral, 35.4 g/kg/52 weeks

Solvent Yellow 14 TDLo, mice, implant, 80 mg/kg, 1968

Trypan Blue TDLo, rats, oral, 440 mg/kg/21 days, 1958 (intermittent dosage)

TDLo, rats, s.c., 1.088 g/kg/87 weeks, 1963 (intermittent dosage)

3. sensitization

Gordon (1964) tested the correlation of carcinogenicity with skin sensitization in a group of butter yellow derivatives. The results are in Table 37. DAB is an abbreviation for 4-amino-N,N-dimethylazobenzene, and MAB is DAB minus one methyl. In column 2, the higher the number the higher the carcinogenicity in rats (not necessarily the same in the guinea pigs used here). The cross-reaction test was run at the same time as the confirmation of sensitivity test; it indicated that an animal sensitized by one of the azo compounds was likely to be sensitized by all. Reaction to the sensitizing compound itself ranged from 100% from a 1% solution of the confirmation dose, to 18% from a 0.001% solution. The desired correlation was not achieved.

Table 37. Sensitizing Capacity and Carcinogenicity of Azo Dyes

Azo dye*	Carcinogenic† Index (rat)	Binding† to rat liver proteins	Capacity‡ to sensitize	Capacity to elicit skin reactions§
DAB	6	+	100 (4)	100 (5)
3'CH_3DAB	10–12	+	100 (5)	75 (4)
3'F DAB	10–12	+	100 (5)	60 (15)
4'CH_3DAB	< 1	+	100 (5)	73 (15)
4'F DAB	10–12	+	100 (5)	80 (15)
MAB	6	+	40 (5)	33 (15)
2 CH_3DAB	0	+	100 (4)	60 (5)
4'NH_2DAB	0	0	0 (5)	75 (8)
3'CF_3DAB	0	0	0 (5)	50 (20)
2 OH DAB	0	0	0 (5)	10 (20)
2'OH DAB	0	0	0 (5)	10 (20)
p-Aminoazobenzene	0	0	0 (5)	5 (20)
Azobenzene	0	0	0 (2)	0 (12)

* For formulæ see text.
† See ref. 2.
‡ The numbers refer to the percentage of animals sensitized; those in brackets indicate the numbers of animals in each experiment.
§ Cross-reactions: the numbers refer to the percentage of animals reacting; those in brackets indicate the number of sensitized animals skin tested.

4. teratogenicity

(Kelley et al, 1964, quoted T. Vickerstaff, 1954, from The Physical Chemistry of Dyeing: "It is virtually impossible to synthesize a pure disazo dye and only slightly easier to purify a given sample." Most of the papers in this section deal with trypan blue, a disazo dye!)

Hamburgh (1952) injected female mice with about 1.7 mg trypan blue one week prior to, and one week past fertilization. Nearly 1/4 of the young showed tail abnormalities. Of these malformed young, 3/4 were males. The experiment was repeated, but the mice were sacrificed 10-14 days after fertilization. There were abnormalities in 60% of the embryos, mostly in the tail and head regions (everted brains). The latter probably resulted in failure to survive to term.

Waddington and Carter (1953) injected female mice in the seventh day of fertilization with 5 mg of trypan blue. In one experiment the young were allowed to be born, in another, the mothers

were sacrificed at one day intervals after injection. Mortality (embryo) rose sharply on the 11th day of gestation. Abnormalities were notably high one day after injection, but decreased sharply on the 11th day. Of the full term animals, litter size was half the normal and males predominated. Head and tail abnormalities predominated.

Hamburgh (1954) reported on a follow-up study in which pregnant mice were injected with 5 mg of Trypan Blue on the 7th day of gestation, and then sacrificed on the 8-14 days of gestation. He found 15% resorption and 60% malformed embryos, mostly head and tail types. The chronology of the malformations is presented in Table 38.

TABLE 38[1]

Incidence of malformation of embryos 8½–14 days after fertilization from mother injected with trypan blue

AGE IN DAYS	TOTAL OF EMBRYOS	NORMALS	ABNORMALS	RESORBED	FREQUENCY OF HEAD ABNORMALITIES	% OF HEAD ABNORMALITIES	FREQUENCY OF TAIL ABNORMALITIES	% OF TAIL ABNORMALITIES	MISC. ABNORMALITIES	% OF MISC. ABNORMALITIES
8½– 9	43	19	23	1	9	20	..	..	13	53
9 – 9½	55	6	41	8	14	25	..	..	34	61
9½–10	81	23	41	17	13	16	..	..	27	33
10½–11	86	21	61	4 ***	13	15	46	53	34	39
11½–12	98	16	62	20 ***	22	22	41	42	41	41
12½–13	155	59	76	20 ***	27	17	57	23	23	14
13½–14	53	20	28	5 ***	8	15	24	45	9	16
Total	571	164	332	75 ***	106	18	149	26	191	33

[1] In breaking down abnormalities into the various categories many cases were listed more than once, whenever more than one type of malformation occurred in the same embryo. This fact should be borne in mind when adding the figures of table 1.

[2] Records of resorbtion are incomplete after the 10th day.

Wilson (1955) gave s.c. injections of 10 mg of a variety of trypan blue-related azo dyes to pregnant rats on the 7th, 8th, and 9th days of gestation. On the 20th day all were sacrificed. Table 39 contains a list of the dyes (o-toluidine is a non-azo ingredient

TABLE 39.

Effects on gestation and the offspring of azo dyes injected on the 7th, 8th, and 9th days of pregnancy

DYE	TOTAL NUMBER MOTHERS	PREGNANCIES TERMINATING BEFORE 20TH DAY		PREGNANCIES CONTINUING TO THE 20TH DAY			
		% maternal death	% complete resorption	% of all pregnancies	Number implantations	% of embryos resorbed	% of survivors malformed
Trypan blue	45	4	16	80	407	44	49
Evans blue	20	10	25	65	162	23	14
Niagara blue 4B	15	7	13	80	126	15	4
Niagara sky blue 6B	15	0	20	80	145	8	3
Congo red 4B	20	30	15	55	123	15	1[1]
Vital red	10	10	70	20	17	12	0
Acid milling red RN	9	11	11	78	78	13	0
Chlorazol black E	16	19	25	56	91	21	0
Benzopurpurin 4B	10	20	10	70	73	19	0
Diamil blue 2R	10	10	0	90	102	10	0
Erie violet 2B	13	0	8	92	148	11	0
Erie garnet B	10	0	0	100	112	7	2[1]
Niagara blue 3 RD	10	0	0	100	115	11	1[1]
O-tolidine	10	0	0	100	109	8	0
Azo blue	6	0	0	100	57	7	0

[1] Hydrocephalus affecting one or two animals in a single litter, not attributed to action of the dyes for reasons described in text.

TABLE 40.

Frequency of malformations in various organs and systems after different dyes

	TOTAL NUMBER LIVING YOUNG	% OF YOUNG MALFORMED	% OF TOTAL LIVING YOUNG SHOWING DEFECTS IN VARIOUS ORGANS AND SYSTEMS				
			Brain	Eye	Cardiovascular	Vertebral column	Other[1]
Trypan blue	228	49	33	10	11	17	12
Evans blue	124	14	13	2	1		1
Niagara blue 4B	107	4	4		1		
Niagara sky blue 6B	134	3	2	1			

[1] Specified in text.

common to the manufacture of the others), the mortality, and teratogenicity found; the mothers had been surgically examined for the number of implants prior to injection, on the 7th day. Table 40 breaks down the malformations according to most frequent type. In the last column of this table, "other" consisted of gastroschisis, short snout, and clubfoot, about equally. The most frequent brain effect was hydrocephalus

accompanied by mesencephalic aqueductal obliteration/constriction. Ocular effects included monolateral (usually) anopthalmia, microphthalmia, and retinal coloboma. Cataract of the lens was also seen, and deemed to be degenerative rather than developmental in nature; its incidence was not known as few animals were examined for it. Cardiovascular effects were (decreasingly): aortal-pulmonary trunk transposition, aortal right-sided arch, aortal double arch, absence of ductus arteriosus, and trunkus arteriosus communis. Vertebral columnar effects were rudimentary-absent lumbar, sacral and caudal vertebrae, absence of entire sacrum, medially displaced ilia. Resultant external and internal changes included trunk shortening, lack of tail, lack of genital/excretal openings, lack of some pelvic viscera.

There was no evidence for the dyes having crossed the placenta. This, together with the occurrence in the same litter of normal appearing (inside and out) embryos at term, did not allow for even an intelligent guess as to the reason for the malformations.

Langman and van Drunen (1959) injected s.c. female rabbits with 5 ml/kg of a 1% trypan blue solution five days prior to, two and seven days post-fertilization. The uterus was excised on the 28th day of gestation. Serum proteins were analyzed at pre- and post-fertilization intervals. Table 41 contains the overall teratogenic statistics. Malformations were found in the spine, tail (none), gut (eventrated), and brain (hydrocephalus). The eyes were normal. Control rabbits showed a decrease in total serum protein and albumin during gestation, but dye-dosed ones showed an increase in both, especially during the first 14 days, followed by a decrease to non-pregnant levels.

The γ-globulin fraction was not increased.

TABLE 41.

Result of pregnancy in 5 normal and 7 trypan blue treated rabbits

	NUMBER OF RABBITS	NUMBER OF IMPLANTED EMBRYOS	NUMBER OF RESORPTIONS AND DEAD FETUSES	NUMBER OF EMBRYOS WITH MALFORMATIONS	NUMBER OF NORMAL EMBRYOS
Normal	**5**	**44**	**1 (2%)**	**0 (0%)**	**43 (98%)**
Trypan blue	**7**	**53**	**21 (39%)**	**9 (17%)**	**23 (44%)**

Beck et al (1960) reported that eight samples of trypan blue from six commercial sources analyzed for only 20-86% pure dye (another sample being too contaminated from another blue dye for meaningful analysis), and concluded that dosage levels previously reported in teratogenic studies were not reliably comparable. The major contaminants were 1-76% NaCl and 5.5-17% H_2O. There was also a red dye present in all eight fully analyzed samples. The ratio of extinction coefficients, E (red)/E (trypan blue), ranged 0.07-0.24.

Pregnant rats (two strains) were given s.c. injections on the 9th day of gestation with either one of the commercial trypan blues (74% actual dye) or various fractions thereof. The results are in Table 42. The purified trypan blue gave the lowest percentage of resorptions. There was no statistical difference in the number of abnormalities seen with the MRC strain.

Table 42.

Substance tested	No. of rats used	Total implantations	Total resorptions	Resorptions (per cent)	External abnormalities in survivors	
					Normal	Abnormal
M.R.C. hooded rats						
Control (uninjected)	14	148	10	6·7	138	0
1 per cent trypan blue (Grübler)	11	117	36	30·7	80	1
1 per cent recrystallized trypan blue	12	144	18	12·5	126	3
1 per cent mother liquor	14	148	46	31·8	102	2
0·5 per cent red impurity	19	224	47	20·9	176	1
Wistar rats						
Control (uninjected)	4	33	2	6·0	31	0
1 per cent crude trypan blue	7	48	27	56·2	9	12

Beaudoin (1961) injected, at 36 hours' incubation, 50 µl of 0.1% dye solution into the subgerminal cavity or 100 µl of 0.1% dye solution into the yolk sac of chicken eggs. Embryos were sacrificed on 10th day. The same was done using trypan blue at 0-96 hours of incubation to test the time span effectiveness of the teratogenicity. The dyes tested in the first study and results are given in Tables 43 and 44.

TABLE 43.

Teratogenic activity of several disazo dyes on chick development when injected at the 36th hour of incubation

Dye	Total treated		Percentage mortality		Percentage malformed survivors			
	Subgerminal	Yolk sac	Subgerminal	Yolk sac	Subgerminal	P values*	Yolk sac	P values
Trypan blue	101	94	45·5	56·5	72·8	< 0·001	56·2	< 0·001
Evans blue	78	90	44·8	14·4	51·3	< 0·001	5·2	0·52
Niagara blue 4B	94	93	69·3	29·9	34·5	0·10	33·4	< 0·001
Niagara blue 2B	67	86	23·9	9·3	27·4	0·47	0	—
Azo blue	82	93	18·3	10·7	20·9	0·90	0	—
Congo red	95	90	59·0	3·3	35·9	0·08	3·4	0·98
Saline controls	220	141	20·9	12·0	20·1	—	2·4	—
Untreated controls	—	134	—	11·9	—	—	3·4	—

* *P* values derived from X^2 test for independence.

TABLE 44.

Frequency of malformations among surviving 10-day chicks (percentage of total survivors)

Dye	Rumpless		Eye		Beak		Gastroschisis		Hind limb		Spina bifida		Other	
	SG*	YS†	SG	YS	SG	YS	SG	YS	SG	YS	SG	YS	SG	YS
Trypan blue	69·1	53·6	7·3	2·7	10·9	—	3·6	—	7·1	4·8	—	—	—	2·4
Evans blue	51·2	2·6	7·0	1·3	2·3	—	4·7	—	9·3	1·3	4·7	—	—	6·5
Niagara blue 4B	20·6	31·8	17·2	3·4	17·2	—	3·4	1·5	3·4	—	—	—	—	4·5
Niagara blue 2B	19·6	—	1·9	—	—	—	—	—	—	—	3·9	—	3·9	—
Azo blue	14·9	—	10·4	—	7·5	—	1·5	—	1·5	—	1·5	—	1·5	—
Congo red	33·4	2·3	12·8	2·3	7·7	—	2·6	—	2·6	—	7·7	—	—	—
Saline controls	9·6	2·4	4·6	0·8	1·5	0·8	4·1	—	1·5	—	8·6	0·8	2·5	—
Untreated controls	—	2·5	—	—	—	0·8	—	0·8	—	—	—	—	—	—

* SG = subgerminal. † YS = yolk sac.

Tables 43 and 44 reprinted with permission from J. Embryol Exptl. Morphol. 9, pt. 1:14-21 (1961). Copyright by Cambridge University Press.

The eye defects were anophthalmia and microphthalmia; beak defects were cross-beak and small beak. The separate trypan blue study indicated insignificant mortality after 48 hours from subgerminal, and 72 hours

from yolk sac injection. Percentage of malformed survivors was unaffected after 72 hours from subgerminal or yolk sac injection. The percentage of malformations regardless of survival peaked at 36 hours after yolk sac, and at 36-48 hours after subgerminal injection.

Beck (1961) obtained three commercial samples of trypan blue and made up solutions of each, 0.01 mole/ml (confirmed by titration of the azo linkage). Pregnant rats were given s.c. injections at 8 1/2 days of gestation of 0.05 mole azo linkage/kg. They were sacrificed at 20 1/2 days of gestation. One of the samples of trypan blue failed to produce any abnormalities, and caused a statistically insignificant increase in percentage of resorptions. The other two samples produced resorptions and abnormalities typical for this rat strain, but in differing amounts. An attempt to see if there was a difference in toxicity between the samples by determining the LD-50 values on non-pregnant females somewhat equivocally indicated that the non-teratogenic sample was also less toxic than the one of the others which was used for comparison.

Hoar and Salem (1961) gave a single injection of 2 ml of 1% trypan blue s.c. to pregnant guinea pigs on a day selected from days 6-13 of gestation. The litters were sacrificed on day 30 of gestation, when it was expected that all organ formation would have been completed (term is 68-70 days normally). There wasn't any pattern during this time span to the reduced weight and reduced crown-rump length seen. Resorptions were highest when the dose was given on day 12 or 13, next highest on day 7. Gross malformations were highest when the dose was given on day 11, day 9 being next. Half of

these malformations were cysts of the anterior thoracic wall, one-third were spina bifida; in decreasing amounts, also seen were microphthalmia, hydrocephaly, edema, and meningocele (all <6%). About half of the small or otherwise abnormal embryos had a posterior cleft palate (possibly merely related to delayed growth).

Some of the gestations had been allowed to go to term. Approximately 1/6th of these resulted in complete resorption, and another 1/4th aborted. The gestations were about one day longer than controls and litter size was smaller (though considerably heavier). These had only 5% abnormalities, all non-fatal.

Izumi (1962) injected six azo compounds, of varying carcinogenicity, into pregnant mice to study the teratogenic effects. There was no apparent correlation of carcino- and teratogenicity. Tables 45-61 present the findings.

Table 45. The development of the fetuses in the control groups with or without intraperitoneal injection of 0.01 ml peanut oil per g of body weight

Group	Day of injection	Total No. of mothers	Total No. of implantations	Fetuses at the 18th day of gestation					No. and types * of each anomalies
				(%) × survived	(%) × resorbed	(%) × dead	(%) △ survivors malformed	(g) body weight mean±standard error	
Not injected		16	96	87 (90.6)	8 (8.3)	1 (1.0)	1 (1.1)	1.16±0.019	1 Pd
Injected	8—9	8	49	47 (95.9)	2 (4.1)	0 (0)	1 (2.1)	1.20±0.016	1 Dt
	10—11	10	74	61 (82.4)	13 (17.6)	0 (0)	0 (0)	1.07±0.015	
	12—13	10	60	43 (71.7)	16 (26.7)	1 (1.7)	1 (2.3)	0.98±0.021	1 Cp
	14—15	7	45	34 (75.6)	10 (22.2)	1 (2.2)	1 (2.9)	1.06±0.016	1 El

* cp: cleft palate, Dt: deviation of toe, El: malformation of elbow joint.
× : % of total No. of implantations △ : % of survived

Table 46. Effects of 3'-trifluoromethyl-4-dimethylaminoazobenzene on the offspring of mice [Intraperitoneal injection of 0.01 ml 5% peanut oil solution (0.5 mg) per g of body weight]

Day of injection	Total No. of mothers	Total No. of implantations	Fetuses at the 18th day of gestation					No. and types of each anomalies
			(%) × survived	(%) × resorbed	(%) × dead	(%) △ survivors malformed	(g) body weight mean±standard error	
8–9	14	107	34 (31.8)	73 (68.2)	0 (0)	1 (2.9)	0.88±0.015	1Dt
10–11	24	149	78 (52.3)	71 (47.7)	0 (0)	15 (19.2)	0.97±0.018	1An, 3Cp, 6Dt, 1El, 2Kn, 3Md, 7Pd
12–13	18	114	57 (50.0)	57 (50.0)	0 (0)	14 (24.6)	1.03±0.018	12Cp, 1Ct, 2Dt
14–15	11	70	64 (91.4)	6 (8.6)	0 (0)	7 (10.9)	1.04±0.018	7Cp

* An: malformation of ankle joint, Cp: cleft palate, Ct: curved tail, Dt: deviation of toe or finger, El: malformation of elbow joint, Kn: malformation of knee joint, Md: macrodactyly of toe, Pd: polydactyly of toe.
× : % of total No. of implantations, △ : % of survived.

Izumi (1962) reported a study which concentrated its effort on changes in fetal bone structure as a result of these dyes being injected into pregnant mice. Tables 62-91 present the results.

Table 47. Effects of monoethylaminoazobenzene on the offspring of mice [Intraperitoneal injection of 0.004 or 0.006ml 5% peanut oil solution (0.2 or 0.3 mg) per g of body weight]

Day of injection	Total No. of mothers	Total No. of implantations	Fetuses at the 18th day of gestation					No. and types* of each anomalies
			(%) × survived	(%) × resorbed	(%) × dead	(%) △ survivors malformed	(g) body weight mean±standard error	
8–9	8	43	40 (93.0)	3 (7.0)	0 (0)	1 (2.5)	1.09±0.020	1 Kn
10–11	18	129	73 (56.6)	55 (42.6)	1 (0.8)	5 (6.8)	1.07±0.018	3 Dt, 1 Kn, 1 Pd
12–13	9	59	36 (61.0)	23 (39.0)	0 (0)	1 (2.8)	0.97±0.033	1 Dt
14–15	7	44	30 (68.2)	14 (31.8)	0 (0)	1 (3.3)	0.91±0.029	1 An, 1 Kn

* An: malformation of ankle joint, Dt: deviation of toe or finger, Kn: malformation of knee joint, Pd: polydactyly of toe. × : % of total No. of implantations, △ : % of survived.

Table 48. Effects of 4'-methyl-4-dimethylaminoazobenzene on the offspring of mice [Intraperitoneal injection of 0.01 ml 5% peanut oil suspension (0.5 mg) per g of body weight]

Day of injection	Total No. of mothers	Total No. of implantations	Fetuses at the 18th day of gestation					No. and types* of each anomalies
			(%) × survived	(%) × resorbed	(%) × dead	(%) △ survivors malformed	(g) body weight mean±standard error	
8–9	8	62	40 (64.5)	22 (35.5)	0 (0)	5 (12.5)	1.19±0.014	1 Dt, 4 Kn, 1 Pd
10–11	10	82	49 (59.8)	33 (40.2)	0 (0)	5 (10.2)	1.23±0.018	3 Dt, 2 Kn
12–13	14	107	71 (66.4)	35 (32.7)	1 (0.9)	3 (4.2)	1.20±0.013	1 Cp, 2 Dt, 1 Kn
14–15	7	57	37 (64.9)	18 (31.6)	2 (3.5)	2 (5.4)	0.95±0.016	1 Ct, 1 Dt

* Cp: cleft palate, Ct: curved tail, Dt: deviation of toe or finger, Kn: malformation of knee joint, Pd: polydactyly of toe.
× : % of total No. of implantations, △ : % of survived.

Table 49. Effects of monomethylaminoazobenzene on the offspring of mice (Intraperitoneal injection of 0.004 or 0.006 ml 5% peanut oil solution (0.2 or 0.3 mg) per g of body weight)

Day of injection	Total No. of mothers	Total No. of implantations	Fetuses at the 18th day of gestation					No. and types* of each anomalies
			(%) × survived	(%) × resorbed	(%) × dead	(%) △ survivors malformed	(g) body weight mean±standard error	
8–9	7	61	40 (65.6)	21 (34.4)	0	4 (10.0)	1.11±0.023	1Dt, 1Kn, 1Md, 2St
10–11	12	81	36 (44.4)	45 (55.6)	0	4 (11.1)	1.03±0.023	1Ad, 1Cp, 1Dt, 1Hl, 1Pd
12–13	19	121	45 (37.2)	76 (62.8)	0	4 (8.9)	1.06±0.013	2An, 1Kn, 1Pd
14–15	7	54	31 (57.4)	23 (42.6)	0	1 (3.2)	1.00±0.019	1Pd

* Ad. adactyly of finger, An: malformation of ankle joint, Cp: cleft palate, Dt: deviation of toe or finger, Hl: harelip, Kn: malformation of knee joint, Md: macrodactyly of toe, Pd: polydactyly of toe, St: short tail.

× : % of total No. of implantations, △ : %of survived.

Table 50. Effects of 3'-fluoro-4-dimethylaminoazobenzene on the offspring of mice (Intraperitoneal injection of 0.006ml 5% peanut oil suspension (0.3mg) per g of body weight)

Day of injection	Total No. of mothers	Total No. of implantations	Fetuses at the 18th day of gestation					No. and types* of each anomalies
			(%) × survived	(%) × resorbed	(%) × dead	(%) △ survivors malformed	(g) body weight mean±standard error	
8–9	9	45	39 (86.7)	6 (13.3)	0 (0)	5 (12.8)	0.92±0.024	1An, 1Dt, 2El, 1Pd
10–11	10	69	25 (36.2)	44 (63.8)	0 (0)	4 (16.0)	1.15±0.030	2Dt, 1El, 2Kn
12–13	9	59	31 (52.5)	27 (45.8)	1 (1.7)	3 (9.7)	0.87±0.034	3An, 1Ct, 1El, 2Kn
14–15	9	57	24 (42.1)	33 (57.9)	0 (0)	1 (4.2)	0.90±0.027	1Cp

* An: malformation of ankle joint, Cp: cleft palate, Ct: curved tail, Dt: deviation of toe or finger, El: malformation of elbow joint, Kn: malformation of knee joint, Pd: polydactyly of toe.

× : % of total No. of implantations, △ : % of survived.

Table 51. Effects of 4'-fluoro-4-dimethylaminoazobenzene on the offspring of mice (Intraperitoneal injection of 0.004 ml 5% peanut oil suspension (0.2 mg) per g of body weight)

Day of injection	Total No. of mothers	Total No. of implantations	Fetuses at the 18th day of gestation					No. and types* of each anomalies
			(%) × survived	(%) × resorbed	(%) × dead	(%) △ survivors malformed	(g) body weight mean±standard error	
8–9	7	55	41 (74.5)	14 (25.5)	0 (0)	3 (7.3)	1.07±0.025	1An, 2Kn, 1Kt
10–11	11	71	28 (39.4)	43 (60.6)	0 (0)	8 (28.6)	1.14±0.023	2An, 4Dt, 1El, 4Kn
12–13	11	85	58 (68.2)	25 (29.4)	2 (2.4)	2 (3.4)	1.00±0.020	1Ct, 1Kn
14–15	7	44	40 (90.9)	4 (9.1)	0 (0)	0 (0)	0.88±0.019	

* An: malformation of ankle joint, Ct: curved tail, Dt: deviation of toe or finger, El: malformation of elbow joint, Kn: malformation of knee joint, Kt. kinky tail.

× : % of total No of implantations, △ : % of survived.

Table 52 Evaluation of the lethal effect of peanut oil injection upon fetuses by χ^2-test : control groups with- and without injection were compared with each other

Day of injection	8—9	10—11	12—13	14—15
	—	—	* *	*

* * : significant at 1 % level,
* : significant at 5 % level,
— : not significant.

Table 53 Evaluation of the growth suppressing effect of peanut oil injection upon fetuses by F-test : control groups with and without injection were compared with each other

Day of injection	8—9	10—11	12—13	14—15
	—	* *	* *	* *

* * : significant at 1 % level,
— : not significant.

Table 54 Evaluation of the lethal effect of various derivatives upon fetuses by χ^2-test compared with the control group treated with the solvent

Derivatives treated / Day of injection	3'-trifluoromethyl-4-dimethylaminoazobenzene	moncethylaminoazobenzene	4'-methyl-4-dimethylaminoazobenzene	monomethylaminoazobenzene	3'-fluoro-4-dimethylaminoazobenzene	4'-fluoro-4-dimethylaminoazobenzene
8— 9	* *	—	* *	* *	—	* *
10—11	* *	* *	* *	* *	* *	* *
12—13	* *	—	—	* *	*	—
14—15	(*)	—	—	—	* *	—

* * : significant at 1 % level, * : significant at 5 % level,
— : not significant, (*) : inverse effect at 5 % significant level.

Table 55 Comparison of mortality between the fetuses treated by various derivatives on the 8th to 9th day

Derivatives treated / Fetuses	3'-trifluoromethyl-4-dimethylaminoazobenzene	4'-methyl-4-dimethylaminoazobenzene	monomethylaminoazobenzene	4'-fluoro-4-dimethylaminoazobenzene	Σ
No. of resorbed or dead	73	22	21	14	130
No. of survived	34	40	40	41	155
Σ	107	62	61	55	285

df = 3, χ^2 = 36.73, P < 0.01

Table 56 Comparison of mortality between the fetuses treated by various derivatives on the 10th to 11th day

Derivatives treated / Fetuses	3'-trifluoromethyl-4-dimethylaminoazobenzene	monoethylaminoazobenzene	4'-methyl-4-dimethylaminoazobenzene	monomethylaminoazobenzene	3'-fluoro-4-dimethylaminoazobenzene	4'-fluoro-4-dimethylaminoazobenzene	Σ
No. of resorbed or dead	71	56	33	45	44	43	292
No. of survived	78	73	49	36	25	28	289
Σ	149	129	82	81	69	71	581

df = 5, χ^2 = 15.13, P < 0.01

Table 57 Comparison of mortality between the fetuses treated by various derivatives on the 12th to 13th day

Derivatives treated / Fetuses	3′-trifluoro-4-dimethylaminoazobenzene	monomethyl-amino-azobenzene	3′-fluoro-4-dimethyl-amino-azobenzene	Σ
No. of resorbed or dead	57	76	28	161
No. of survived	57	45	31	133
Σ	114	121	59	294

df = 2, $\chi^2 = 5.44$, $P > 0.05$

Table 58 Evaluation of the teratogenic effect of various derivatives upon fetuses by χ^2-test compared with the control group treated with the solvent (some cases are calculated by exact method)

Derivatives treated / Day of injection	3′-trifluoromethyl-4-dimethylaminoazobenzene	monoethylaminoazobenzene	4′-methyl-4-dimethylaminoazobenzene	monomethylaminoazobenzene	3′-fluoro-4-dimethylaminoazobenzene	4′-fluoro-4-dimethylaminoazobenzene
8 – 9	—	—	—	—	—	—
10 – 11	* *	*	*	*	* *	* *
12 – 13	*	—	—	—	—	—
14 – 15	—	—	—	—	—	—

* * : significant at 1 % level,
* : significant at 5 % level,
— : not significant.

Table 59 Comparison of teratogenicity between the fetuses treated by various derivatives on the 10th to 11th day

Derivatives treated / Fetuses	3′-trifluoromethyl-4-dimethylaminoazobenzene	monoethylaminoazobenzene	4′-methyl-4-dimethyl aminoazobenzene	monomethylaminoazobenzene	3′-fluoro-4-dimethylaminoazobenzene	4′-fluoro-4-dimethylaminoazobenzene	Σ
Malformed	15	5	5	4	4	8	41
Normal	63	68	44	32	21	20	248
Σ	78	73	49	36	25	28	289

df = 5, $\chi^2 = 10.56$, $P > 0.05$

Table 60 Evaluation of the growth suppressing effect of various derivatives upon fetuses by F-test compared with the control group treated with the solvent

Derivatives treated / Day of injection	3′-trifluoromethyl-4-dimethylaminoazobenzene	moncethylaminoazobenzene	4′-methyl-4-dimethylaminoazobenzene	monomethylaminoazobenzene	3′-fluoro-4-dimethylamino-azobenzene	4′-fluoro-4-dimethylaminoazobenzene
8 – 9	* *	* *	—	* *	* *	* *
10 – 11	* *	—	(* *)	—	(*)	(*)
12 – 13	—	—	(* *)	(* *)	* *	—
14 – 15	—	* *	* *	*	* *	* *

* * : significant at 1 % level, * : significant at 5 % level, — : not significant,
(*) : inverse effect at 5 % significant level, (* *) : inverse effect at 1 % significant level.

Table 61. Comparison of the growth suppressing effect between the malformed and normal fetuses treated by various derivatives on the 10th to 13th day by F-test

Derivatives treated / Day of injection	3'-trifluoromethyl-4-dimethylaminoazobenzene	monoethylaminoazobenzene	4'-methyl-4-dimethylaminoazobenzene	monomethylaminoazobenzene	3'-fluoro-4-dimethylaminoazobenzene	4'-fluoro-4-dimethylaminoazobenzene
10—11	—	—	(-)	—	—	—
12—13	—					

— : not significant,
(-) : inverse effect at 1 % significant level.

Table 62. Occurrence of malformed ribs in the offspring of control mice treated with peanut oil

Day of injection	8—9	10—11	12—13	14—15
No. of mothers	8	2	2	5
No. of fetuses	47	11	10	26
No. of fetuses with malformed ribs	0	0	0	0

Table 63. Occurrence of malformed ribs in the offspring of mice treated with 4'-methyl-4-dimethylaminoazobenzene
[0.5mg/g (body weight)]

Day of injection	8—9	10—11	12—13	14—15
No. of mothers	5	7	11	6
No. of fetuses	40	49	71	37
No. of fetuses with malformed ribs	1	1	0	0

— : not significant.

Table 64. Occurrence of malformed ribs in the offspring of mice treated with monomethylaminoazobenzene
[0.2 or 0.3 mg/g (body weight)]

Day of injection	8—9	10—11	12—13	14—15
No. of mothers	5	6	8	5
No. of fetuses	40	36	45	31
No. of fetuses with malformed ribs	5 *	0	0	1

* : significant at 5 % level,
— : not significant.

Table 65. Occurrence of malformed ribs in the offspring of mice treated with 3'-fluoro-4-dimethylaminoazobenzene
[0.3 mg/g (body weight)]

Day of injection	8—9	10—11	12—13	14—15
No. of mothers	5	5	6	5
No. of fetuses	39	25	31	24
No. of fetuses with malformed ribs	5 *	0	0	0

* : significant at 5 % level.

Table 66. Occurrence of malformed ribs in the offspring of mice treated with 4'-fluoro-4-dimethylaminoazobenzene [0.2 mg/g (body weight)]

Day of injection	8—9	10—11	12—13	14—15
No. of mothers	5	6	9	7
No. of fetuses	41	28	58	40
No. of fetuses with malformed ribs	1	0	0	1

— : not significant.

Table 67. Classification of fetuses according to No. of ossified sternebrae in control mice treated with peanut oil

No. of stained sternebrae \ Day of injection	8—9	10—11	12—13	14—15
0—2	0	0	0	0
3—4	0	0	0	0
5—6	47	11	10	26
Total	47	11	10	26

Table 68. Classification of fetuses according to No. of ossified sternebrae in mice treated with 4'-methyl-4-dimethylaminoazobenzene [0.5 mg/g (body weight)]

No. of stained sternebrae \ Day of injection	8—9	10—11	12—13	14—15
0—2	— 0	0	— 1	0
3—4	1	0	0	0
5—6	39	49	70	37
Total	40	49	71	37

— : not significant.

Table 69. Classification of fetuses according to No. of ossified sternebrae in mice treated with monomethylaminoazobenzene [0.2 or 0.3 mg/g (body weight)]

No. of stained sternebrae \ Day of injection	8—9	10—11	12—13	14—15
0—2	* 1	— 0	— 0	0
3—4	5	1	1	0
5—6	34	35	44	31
Total	40	36	45	31

* : : significant at 5 % level,
— : not significant.

Table 70. Classification of fetuses according to No. of ossified sternebrae in mice treated with 3'-fluoro-4-dimethylaminoazobenzene [0.3mg/g (body weight)]

No. of stained sternebrae \ Day of injection	8—9	10—11	12—13	14—15
0—2	+ 1	0	— 2	0
3—4	3	0	0	0
5—6	35	25	29	24
Total	39	25	31	24

+ : significant at 10% level,
— : not significant.

Table 71. Classification of fetuses according to No. of ossified sternebrae in mice treated with 4'fluoro-4-dimethylaminoazobenzene [0.2 mg/g (body weight)]

No. of stained sternebrae \ Day of injection	8—9	10—11	12—13	14—15
0—2	— 0	— 0	0	— 0
3—4	1	1	0	3
5—6	40	27	58	37
Total	41	28	58	40

— : not significant.

Table 72. No. of fetuses with mislocated ossification centers of sternebrae in control mice treated with peanut oil

Day of injection	8 — 9	10—11	12—13	14—15
Mislocated	1	0	0	0
Normally located	46	11	10	26
Total	47	11	10	26

Table 73. No. of fetuses with mislocated ossification centers of sternebrae in mice treated with 4'-methyl-4-dimethylaminoazobenzene
〔0.5mg/g (body weight)〕

Day of injection	8 — 9	10—11	12—13	14—15
Mislocated	— 3	— 7	— 2	— 2
Normally located	37	42	69	35
Total	40	49	71	37

— : not significant.

Table 74. No. of fetuses with mislocated ossification centers of sternebrae in mice treated with monomethylaminoazobenzene
〔0.2 or 0.3mg/g (body weight)〕

Day of injection	8 — 9	10—11	12—13	14—15
Mislocated	+ 6	* 10	— 3	— 2
Normally located	34	26	42	29
Total	40	36	45	31

* : significant at 5 % level,
+ : significant at 10% level,
— : not significant.

Table 75. No. of fetuses with mislocated ossification centers of sternebrae in mice treated with 3'-fluoro-4-dimethylaminoazobenzene
〔0.3mg/g (body weight)〕

Day of injection	8 — 9	10—11	12—13	14—15
Mislocated	8	— 1	— 2	— 2
Normally located	31	24	29	22
Total	39	25	31	24

* : significant at 5 % level,
— : not significant.

Table 76. No. of fetuses with mislocated ossification centers of sternebrae in mice treated with 4'-fluoro-4-dimethylaminoazobenzene
〔0.2mg/g (body weight)〕

Day of injection	8 — 9	10—11	12—13	14—15
Mislocated	— 4	— 3	— 2	— 4
Normally located	37	25	56	36
Total	41	28	58	40

— : not significant.

Table 77 No. of fetuses with not ossified 13th rib in control mice treated with peanut oil

Day of injection right (r) or left (l) / Staining of the 13th rib	8—9 r.	8—9 l.	10—11 r.	10—11 l.	12—13 r.	12—13 l.	14—15 r.	14—15 l.
Not stained	1	1	0	0	0	0	3	4
	2	1	0	0	0	0	4	4
Faintly stained	1	0	0	0	0	0	1	0
Stained	45	46	11	11	10	10	22	22
Total	47	47	11	11	10	10	26	26

Table 78 No. of fetuses with not ossified 13th rib in mice treated with 4'-methyl-4-dimethylaminoazobenzene

〔0.3 mg/g (body weight)〕

Day of injection right (r) of left (l) / Staining of the 13th rib	8—9 r.	8—9 l.	10—11 r.	10—11 l.	12—13 r.	12—13 l.	14—15 r.	14—15 l.
Not stained	13	13	10	7	6	6	1	0
	** 15	** 16	— 13	— 10	— 9	— 8	— 4	— 4
Faintly stained	2	3	3	3	3	2	3	4
Stained	25	24	36	39	62	63	33	33
Total	40	40	49	49	71	71	37	37

** : significant at 1% level, — : not significant.

Table 79 No. of fetuses with not ossified 13th rib in mice treated with monomethylaminoazobenzene

〔0.2 or 0.3mg/g (body weight)〕

Day of injection right (r) of left (l) / Staining of the 13th rib	8—9 r.	8—9 l.	10—11 r.	10—11 l.	12—13 r.	12—13 l.	14—15 r.	14—15 l.
Not stained	6	7	3	2	4	3	1	2
	+ 7	** 11	— 6	— 3	— 8	— 6	— 2	— 2
Faintly stained	1	4	3	1	4	3	1	0
Stained	33	29	30	33	37	39	29	29
Total	40	40	36	36	45	45	31	31

** : significant at 1% level, + : significant at 10% level, — : not significant.

Table 80 No. of fetuses with not ossified 13th rib in mice treated with 3' fluoro-4-dimethylaminoazobenzene

〔0.3mg/g (body weight)〕

Day of injection right (r) or left (l) / Staining of the 13th rib	8—9 r.	8—9 l.	10—11 r.	10—11 l.	12—13 r.	12—13 l.	14—15 r.	14—15 l.
Not stained	3	3	3	0	2	1	3	2
	— 3	— 4	— 4	0	— 2	— 1	— 3	— 2
Faintly stained	0	1	1	0	0	0	0	0
Stained	36	35	21	25	29	30	21	22
Total	39	39	25	25	31	31	24	24

— : not significant.

Table 81. No. of fetuses with not ossified 13th rib in mice treated with 4'-fluoro-4-dimethylaminoazobenzene

〔0.2mg/g (body weight)〕

Day of injection / right(r) or left (l)	8—9		10—11		12—13		14—15	
Staining of the 13th rib	r.	l.	r.	l.	r.	l.	r.	l.
Not stained	2	2	0	0	0	0	3	2
	— 2	— 2	0 — 0	0 — 0	— 1	0 — 0	— 3	— 2
Faintly stained	0	0	0	0	1	0	0	0
Stained	39	39	28	28	57	58	37	38
Total	41	41	28	28	58	58	40	40

— : not significant.

Table 82. Classification of fetuses according to No. of ossified caudal vertebrae in control mice treated with peanut oil

Day of injection / No. of stained caudal vertebrae	8—9	10—11	12—13	14—15
0—1	0	0	0	0
2—4	5	3	7	8
5—7	42	8	3	18
Total	47	11	10	26

Table 83. Classification of fetuses according to No. of ossified caudal vertebrae in mice treated with 4'-methyl-4-dimethylaminoazobenzene

〔0.5mg/g (body weight)〕

Day of injection / No. of stained caudal vertebrae	8—9	10—11	12—13	14—15
0—1	** 0	— 0	(**) 0	** 0
2—4	22	31	15	36
5—7	17	18	56	1
Total	40	49	71	37

** : significant at 1% level,
— : significant at 10% level,
(**) : inverse effect at 1% significant level.

Table 84. Classification of fetuses according to No. of ossified caudal vertebrae in mice treated with monomethylaminoazobenzene

〔0.2 or 0.3 mg/g (body weight)〕

Day of injection / No. of stained caudal vertebrae	8—9	10—11	12—13	14—15
0—1	** 2	— 0	— 1	** 0
2—4	17	25	25	23
5—7	21	11	19	8
Total	40	36	45	31

** : significant at 1% level,
* : significant at 5% level,
— : not significant.

Table 85. Classification of fetuses according to No. of ossified caudal vertebrae in mice treated with 3'-fluoro-4-dimethylaminoazobenzene

〔0.3mg/g (body weight)〕

Day of injection / No. of stained caudal vertebrae	8—9	10—11	12—13	14—15
0—1	** 3	— 0	— 6	** 2
2—4	24	14	20	21
5—7	12	11	5	1
Total	39	25	31	24

** : significant at 1% level,
— : not significant.

Table 86. Classification of fetuses according to No. of ossified caudal vertebrae in mice treated with 4'-fluoro-4-dimethylaminoazobenene

〔0.2 mg/g (body weight)〕

Day of injection / No. of stained caudal vertebrae	8—9	10—11	12—13	14—15
0 — 1	** 0	— 0	— 0	** 3
2 — 4	27	13	39	30
5 — 7	14	15	19	7
Total	41	28	58	40

* : significant at 1 % level,
— : not significant.

Table 87. Classification of fetuses according to No. of ossified metacarpal and phalangeal bones in the hand or ossified metatarsal and phalangeal bones in the foot in control mice treated with peanut oil

Day of injection / Total No. of stained bones		8—9	10—11	12—13	14—15
Hand	0— 5	0	0	2	0
	6—11	12	0	4	5
	12—17	35	11	4	21
Foot	0— 5	1	0	4	0
	6—11	11	0	3	6
	12—17	35	11	3	20
Total		47	11	10	26

Table 88. Classification of fetuses according to No. of ossified metacarpal and phalangeal bones in the hand or ossified metatarsal and phalangeal bones in the foot in mice treated with 4'-methyl-4-dimethylamino-azobenene

〔0.5mg/g (bydy weight)〕

Day of injection / Total No. of stained bones		8—9	10—11	12—13	14—15
Hand	0— 5	(*) 0	— 0	(**) 0	— 2
	6—11	2	9	2	14
	12—17	38	40	49	21
Foot	0— 5	— 2	* 2	(**) 2	** 7
	6—11	5	20	0	20
	12—17	33	27	69	10
Total		40	49	71	37

* : significant at 1 % level, * : significant at 5 % level, — : not significant,
(*) : inverse effect at 5 % significant level,
(**) : inverse effect at 1 % significant level.

Table 89. Classification of fetuses according to No. of ossified metacarpal and phalangeal bones in the hand or ossified metatarsal and phalangeal bones in the foot in mice treated with monomethylaminoazobenzene

〔0.2or 0.3mg/g (body weight)〕

Day of injection / Total No. of stained bones		8—9	10—11	12—13	14—15
Hand	0— 5	* 5	* 1	(**) 1	— 1
	6—11	9	13	2	8
	12—17	26	22	42	22
Foot	0— 5	** 9	* 8	(**) 1	* 3
	6—11	11	9	12	14
	12—17	20	19	32	14
Total		40	36	45	31

** : significant at 1 % level, * : significant at 5 % level, — : no. significant, (**) : inverse effect at 1 % significant level.

Table 90. Classification of fetuses according to No.of ossified matacarpal and phalangeal bones in the hand or ossified metatarsal and phalangeal bones in the foot in mice treated with 3'-fluoro-4-dimethylamino-azobenzene

〔0.3 mg/g (body weight)〕

Day of injection / Total No. of stained bones		8—9	10—11	12—13	14—15
Hand	0— 5	** 12	** 0	— 10	** 6
	6—11	16	11	14	8
	12—17	11	11	7	10
Foot	0— 5	** 17	** 5	— 14	** 10
	6—11	11	9	13	13
	12—17	8	11	4	1
Total		39	25	31	24

** : significant at 1 % level,
— : not significant.

Table 91. Classification of fetuses according to No. of ossified metacarpal and phalangeal bones in the hand or ossified metatarsal and phalangeal bones in the foot in mice treated with 4'-fluoro-4-dimethylaminoazo-benzene

〔0.2 mg/g (body weight)〕

Day of injection / Tatal No. of stained bones		8—9	10—11	12—13	14—15
Hand	0— 5	+ 4	— 2	— 8	— 3
	6—11	13	3	20	11
	12—17	21	23	30	26
Foot	0— 5	** 10	— 3	— 19	* 8
	6—11	6	3	16	14
	12—17	25	22	23	18
Total		41	28	58	40

** : significant at 1 % level, * : significant at 5 % level, + : significant at 10% level,
— : not significant.

Kelly et al (1964) acquired eight samples of commercial Trypan Blue, including a pair especially prepared to contain extra "red" contaminant. Half gram units of the as-received dye were made up to 50 ml in 0.9% saline. Other 1/2 g units were Soxhlet extracted; the thimble residue, mostly blue dye, was made up to 50 ml, and the extractant, mainly red dye with very little blue, evaporated down and made up to 50 ml in saline. No attempt was made to adjust the whole, blue, or red solutions from the various suppliers to the same "concentration." On each of days 8, 9, and 10 of gestation, rats were given 1 ml i.p. injections of one or the other of these three test solutions. They were sacrificed on day 20. Resorption and malformation statistics are given in Table 92. The malformed fetuses showed reduced body size, edema, exencephaly, spina bifida, but rare caudal defects.

The teratogenic action of the whole dyes did not correlate with actual dye content (53-82% blue, 3.6-13.7% red--the two special lots, dyes 7 and 8, had 22 and 35% red).

Lloyd and Beck (1966) purified some commercial dyes and one especially-prepared dye, all related to trypan blue: Afridol blue (91%), Evans blue (100%), Niagara blue 2B (90%), and Niagara blue 4B (91%). Rats were given s.c. injections of 1% aqueous solutions at 8.5 days of gestation, and then sacrificed at 20.5 days. Variations of treatment involved sacrifice of Niagara blue 4B-treated rats at 11.5 and 14.5 days, and sacrificing Evans blue 7.5-day injected rats at 20.5 days.

It had been determined that both sexes of this rat strain responded the same with respect to serum dye levels after

injection. A number of male rats were given s.c. doses of the 1% solutions; their serum dye levels were determined at intervals of 12 hours (each determination required a different rat as sacrifice was involved).

TABLE 92.

EFFECTS OF TRYPAN BLUE ON FETUSES

Dye	No. litters	No. fetuses	Litter size (av.)	Anomalous individuals No.	Anomalous individuals %	Resorptions No.	Resorptions %
Whole dyes							
1	5	38	7.6	12	31.6	12	24.0
2	3	25	8.3	8	32.0	6	19.4
3	3	29	9.7	11	37.9	2	6.5
4	6	31	5.2	17	54.8	19	37.0
5	3	29	9.7	3	10.3	4	12.1
6	3	33	11.0	1	3.0	0	0.0
7	3	29	9.7	1	3.4	0	0.0
8	3	28	9.3	3	10.7	4	12.5
	29	242	8.8	56	23.1	47	16.3
Blue fractions							
1	5	35	7.0	5	14.3	9	20.4
2	4	29	7.3	3	10.3	4	12.1
3	2	16	8.0	0	0.0	1	5.9
4	3	22	7.3	1	4.5	12	35.3
5	4	30	7.5	0	0.0	0	0.0
6	3	13	4.3	4	30.8	20	60.6
7	2	11	5.5	0	0.0	9	45.0
8	3	30	10.0	0	0.0	1	3.2
	26	186	7.1	13	7.0	56	23.1
Red fractions							
1	3	26	7.7	1	4.1	6	20.7
2	3	27	9.0	0	0.0	0	0.0
3	3	32	10.7	1	3.1	4	11.1
4	6	40	6.7	1	2.5	5	11.1
5	3	27	9.0	0	0.0	2	6.9
6	3	34	11.3	2	5.9	0	0.0
7	2	23	11.5	2	8.7	0	0.0
8	3	31	10.3	1	3.2	0	0.0
	26	237	9.5	8	3.4	17	6.7
All dyes	81	665	8.5	77	11.6	120	15.3
Controls	16	170	10.6	3	1.8	2	1.2

Resorption-abnormality statistics are given in Table 93.

Figures 5, 6, and 7 present maternal deaths as a function of dosage, resorption-abnormality as a function of dosage, and serum level changes

with time--all including trypan blue for comparison, results previously reported. For comparison, in regards to Figure 5, the authors determined LD-50 values on males over a 12-day period, and found 179 mg/kg for Niagara blue 4B and >400 mg/kg for Afridol blue.

Table 9. *Teratogenic response to four bisazo dyes injected subcutaneously at 8·5 days of pregnancy*

Dye	Dose (mg/kg)	No. of mothers: Injected	No. of mothers: Surviving to term	Total implantations	Resorbed: No.	Resorbed: %*	Abnormal: No.	Abnormal: %*	Normal: No.	Normal: %*
Niagara blue 2B	50	11	11	97	7	9·0	1	0·9	89	90·1
	100	13	12	114	33	28·7	4	3·5	77	67·8
	150	14	12	128	46	40 7	32	22·2	50	37·1
	200	7	5	53	53	100	0	0	0	0
Niagara blue 4B	50	13	13	122	31	33·5	3	2·4	88	64·1
	75	12	11	109	39	36·4	0	0	70	63·6
	100	13	11	114	64	56·6	0	0	50	43·4
	150	11	5	50	50	100	0	0	0	0
Afridol blue	25	10	10	123	22	17·2	9	7·6	92	75·2
	50	14	14	134	32	26·0	11	7·7	91	66·3
	100	8	8	77	16	21·8	13	16·3	48	61·9
	150	11	11	113	57	50·3	21	17·2	35	32·5
	200	10	9	80	45	58·7	10	13·2	25	28 1
	300	9	8	86	71	81·7	7	10·6	8	7·7
Evans blue	50	7	7	70	13	16·3	1	1·1	56	82·6
	100	8	8	72	31	48·4	2	2·5	39	49 1
	150	8	7	70	66	95·2	4	4 8	0	0
	200	10	7	74	58	79·8	2	2·4	14	17·9

* Percentages represent the arithmetic means of the percentage within each individual litter; this enables the standard error of the mean resorptions and malformations for each dose to be calculated and shown in Fig. 6.

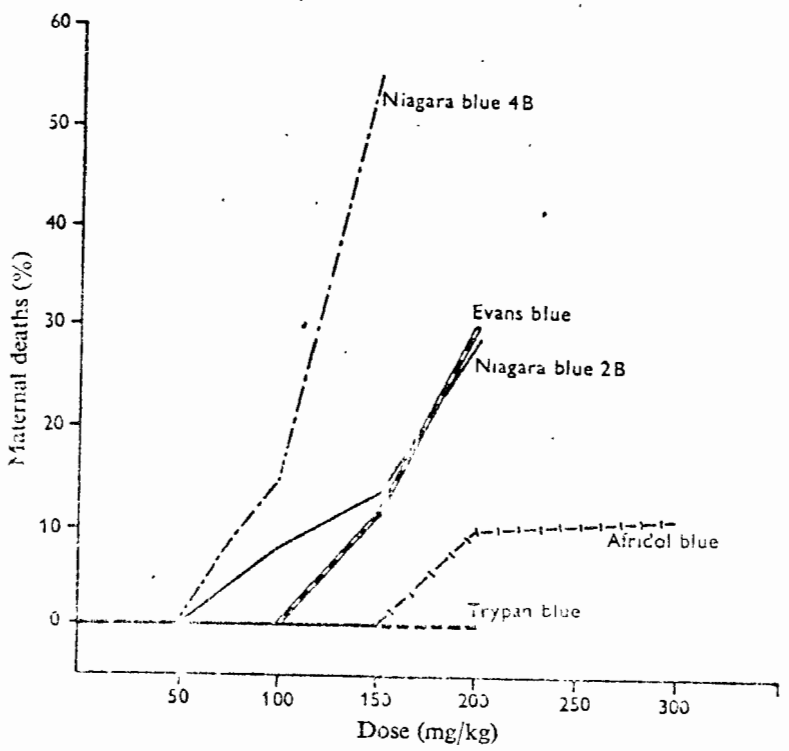


Fig. 5. Maternal mortality within 12 days of administration of various bisazo dyes.

Figures 5-7 reprinted with permission from J. Embrol. Exptl. Morphol. 16:29-39 (1966). Copyright by Cambridge University Press.

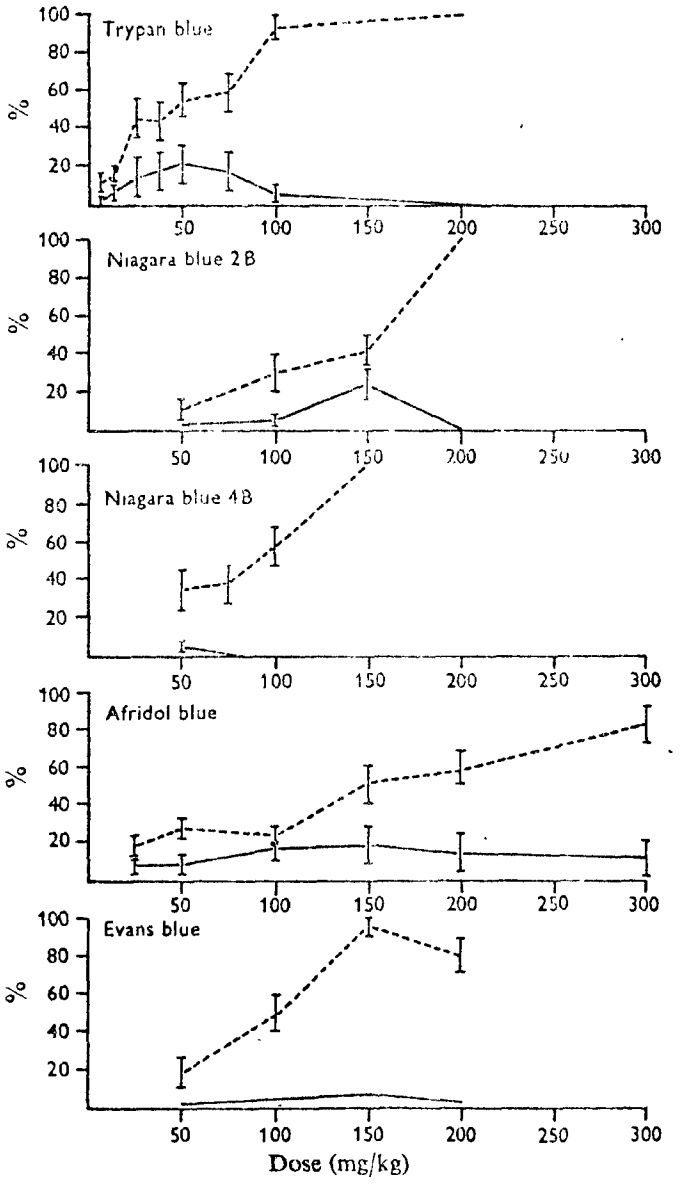


Fig. 6. Dosage–response curves for the teratogenic activity of five bisazo dyes. – – –, Resorptions; – · – · –, abnormalities.

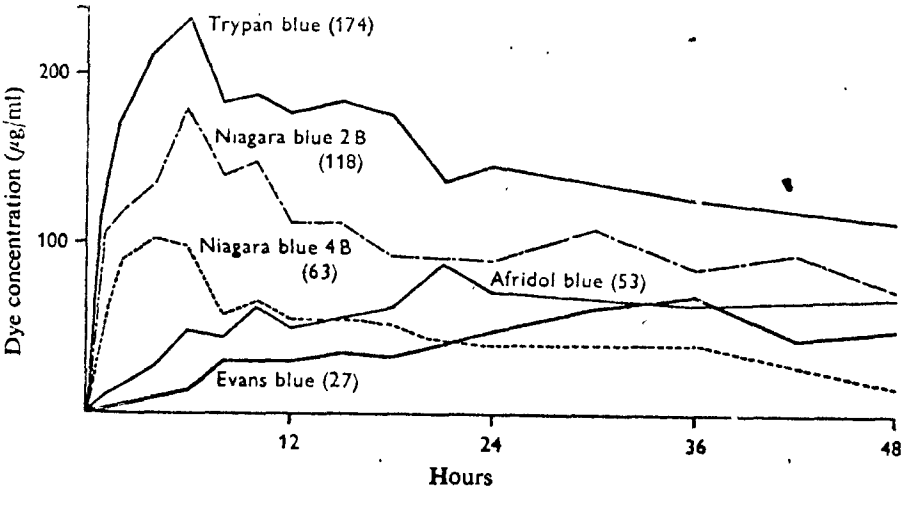


Fig. 7. Serum levels following injection of 50 mg/kg of various dyes (figures in parenthesis are the average levels over the first 24 h).

The reason for giving an Evans blue injection at day 7.5 was the very slow release into the blood stream from the injection site, and a high enough dose to get results comparable with the other dyes would be too toxic to the mother. Thus, injections of 100 or 150 mg/kg at day 7.5 produced these changes from the same doses at day 8.5: at 100 mg/kg % resorptions dropped to 30, % abnormalities rose to 12.7; at 150 mg/kg % abnormalities dropped to 0.

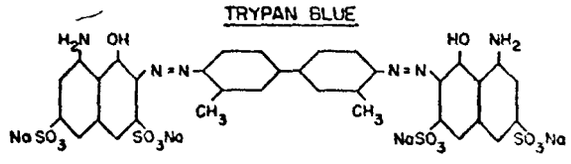
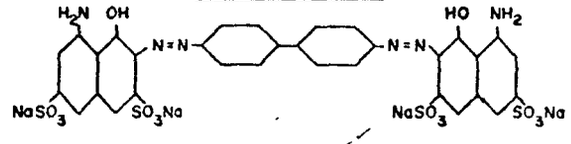
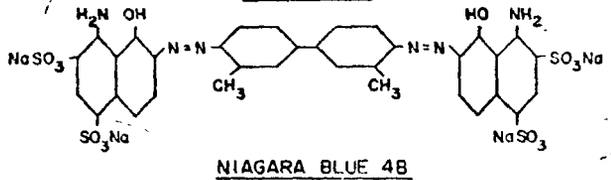
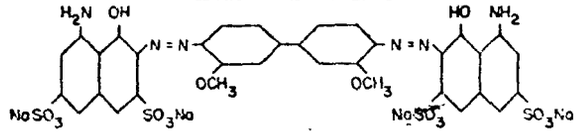
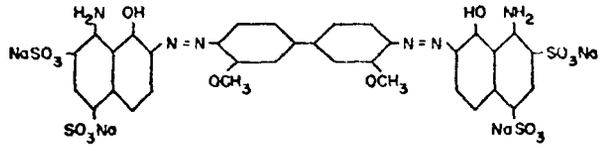
The results of killing the mothers at 11.5 and 14.5 days after a 100 mg/kg dose of blue 4B at 8.5 days indicated most of the resorptions occurred by 11.5 days, presumably from toxicity rather than secondary consequences of malformation.

Beaudoin and Pickering (1966) synthesized 16 dyes related in some fashion to trypan blue and gave them as 140 mg/kg i.p. injections to 8-day pregnant rats, which were sacrificed on the 20th day. Autopsy samples of the maternal macrophage system, kidneys, placenta, and yolk sac were examined for the presence of the injected dye.

Table 94 presents dose-comparable literature results on some highly relevant compounds. Table 95 presents the five compounds most closely related to trypan blue structurally, and results. Of the remaining 11 dyes, none was shown to be a teratogen, nor was there any found in the tissues examined--six of these dyes consisted of simulations of the compounds in Table 94 cleaved at the biphenyl linkage. Table 96 presents the tissue distribution of the compounds whose structures were given. The authors disclaimed Compound 1 as a teratogen, and were reluctant to so label Compound 8, pending further study.

TABLE 94.

Teratogenic activity of disazo dyes in the rat

	Number of mothers	Number of implantations	Embryos resorbed	Survivors malformed
			%	%
TRYPAN BLUE 	19	181	57	54
NIAGARA BLUE 2B 	16	165	23	0
EVANS BLUE 	20[1]	162	23	14
NIAGARA BLUE 4B 	15[1]	126	15	4
NIAGARA SKY BLUE 6B 	15[1]	145	8	3

[1] Data from Wilson, '55.

Tables 94-96 reprinted with permission from Anat. Rec. 137:297-305 (1966). Copyright by Wistar Institute Press.

TABLE 95.

Effects of synthesized disazo compounds on rat gestation

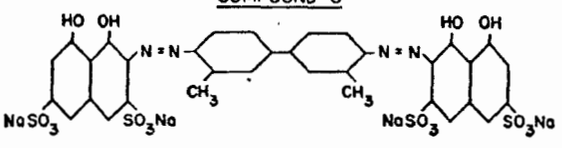
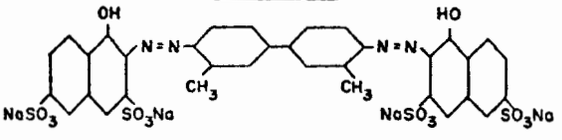
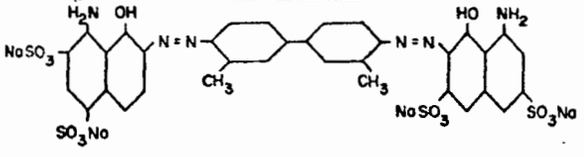
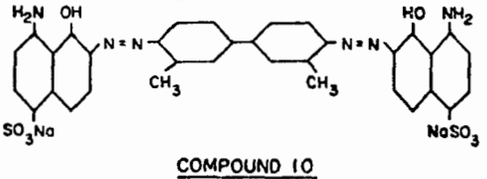
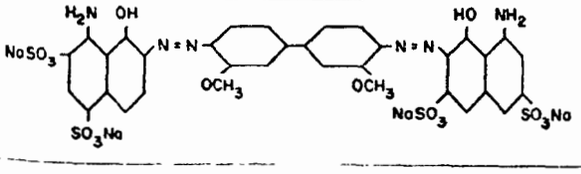
	Number of mothers	Number of implantations	Embryos resorbed	Survivors malformed
			%	%
COMPOUND 6 	7	69	22	0
COMPOUND 1 	6	48	4	2
COMPOUND 8 	8	76	14	3
COMPOUND 5 	5	53	0	0
COMPOUND 10 	6	60	7	0

TABLE 96.

Tissue distribution of disazo dyes and selected synthetic compounds

	Maternal macrophage cells of liver, spleen, lymph node and lung	Maternal kidney	Yolk sac epithelium
Trypan blue	++	+++	+++
Niagara blue 2B	++	+++	+++
Evans blue	++	+++	+++
Niagara blue 4B	++	+++	++
Niagara sky blue 6B	−+	−+	+
Compound 6	+	−	−
Compound 1	−	−	−
Compound 8	+	+++	+++
Compound 5	−	−	−
Compound 10	+	++	+++

Pizzarello and Ford, Jr. (1968) dissolved 6 mg of 4-dimethylaminoazobenzene in 0.1 ml of polyethylene glycol or PEG/ethanol (9/1) and injected it through the shell and air space into the yolk of 2-day old chicken eggs. From the PEG injection all of the surviving chicks had shortened leg bones, and half had deformed feathers. From the PEG/ethanol injection most had shortened bones, and 60-70% had deformed feathers.

Stein et al (1969) injected 10 μg of Janus green B into the amniotic fluid of incubated eggs at the 29 Hamburger-Hamilton stage. All of the survivors exhibited syndactylism.

5. carcinogenicity

Reports of tumors resulting from repetitive injections at the same site have not been included unless the tumors appeared other than at the injection site. Reports dealing with anti-cancer testing of azo compounds, and any metabolic-physiologic information contained therein have been incorporated into this carcinogenic reports section.

Seligman et al (1952) found that growth of sarcoma 37 in mice and Walker carcinoma in rats was inhibited by 1-methyl-2-(phenylazo)naphthalene and 1,4-dimethyl-2-(phenylazo)naphthalene. Sarcoma 37, only, was inhibited by 3-phenylazophenanthrene, 2,2',5,5'- and 3,4,4',5-tetramethoxyazobenzene. Walker carcinoma, only, was inhibited by 3-phenylazoacenaphthene, 3,3'-dimethylazobenzene, and 3,3',4,4',5,5'-hexamethoxyazobenzene.

Simpson (1952) gave rats s.c. injections of 10 mg of Trypan Blue every two weeks for 14-16 weeks in some, much longer in others. Of those given the continuous dose and surviving for 151-250 days, 12 of

21 had hepatic reticulum cell sarcomas; 6 of 9 surviving 251-350 days had this tumor. Of those given 14 doses in 182 days and surviving 210-250 days, 3 of 5 had tumors. The author was unable to demonstrate the transplantability of the tumors in 24 attempts, but may not have allowed sufficient observation time. He was aware of the impure nature of the commercial dye and was not at all certain that the trypan blue component was responsible for the tumors, in part or in whole.

Miller et al (1953) fed rats 4-dimethylaminoazobenzene (DAB) with 1, 2, or 3 fluoro groups in the non-amino benzene ring for comparison of carcinogenicity with DAB itself. The results, in Table 97, were interpreted as meaning that the carcinogenicity of DAB did not involve any of the o-, m-, or p-positions of the non-amino ring. Also tested, and also found to be more carcinogenic than DAB, was 2-fluoro-DAB (Series II, Group 6).

TABLE 97.

THE CARCINOGENICITIES OF VARIOUS FLUORO DERIVATIVES OF 4-DIMETHYLAMINOAZOBENZENE

Series	Group	Compound fed	Per cent in diet	Time compound was fed (mo.)	Incidence of liver tumors* (mo.) 3	4	5	6	Gross cirrhosis at end of feeding compound
I	1	DAB	0.054	3	2/15		7/15		none-mild
	2	2'-Fluoro-DAB	0.059	"	4/13		8/15		mild
	3	3'- " "	"	"	8/14		12/14		moderate
	4	4'- " "	"	"	16/25		24/25		"
II	5	DAB	0.054	3	2/16		5/16		none-mild
	6	2-Fluoro-DAB	0.059	"	3/15		13/15		mild-moderate
	7	2',4'-Difluoro-DAB	0.063	"	10/16		16/16		moderate-severe
III	8	DAB	0.054	4		3/16		11/16	none-mild
	9	2',5'-Difluoro-DAB	0.063	3	9/16		16/16		moderate
	10	3',5'- " "	"	"	9/14		14/14		"
IV	11	DAB	0.054† −0.045	3	1/15		6/15		none-mild
	12	2',4',6'-Trifluoro-DAB	0.066† −0.049	"	5/15		13/15		moderate-severe
V	13	DAB	0.06	4		2/15		7/15	none-mild
	14	" + sodium fluoroacetate	0.06† 0.002	"		3/15		7/15	" "
	15	Sodium fluoroacetate	0.002	10				0/16 (and at 10 mos.)	none

* No. animals with tumors/number of animals alive at end of dye feeding.

Groups 11 and 12 received the high "% in diet" only for the first week, that level of the trifluoro-DAB proving too toxic, and it being desirable to treat the DAB controls the same as the test group so far as molar amount of the dye given. Series V was an attempt to determine the carcinogenicity of a possible metabolite of the fluoro groups, fluoroacetate, but it was too toxic to be given at the maximum potential level; it didn't show any carcinogenicity at the maximum level tolerable to the rats.

Nelson and Woodard (1953) fed dogs o-aminoazotoluene (AAT) or 4-dimethylaminoazotoluene (DAB). The dose of 20 mg/kg/day of AAT killed all the animals within eight weeks from hepatic damage (no tumors). The same dose of DAB killed 8/10 dogs in 16 months (no tumors), the remaining two having tumors. At 5 mg/kg/day AAT produced no tumors in four months in one dog, and tumors in four dogs in 30-62 months. The same dose of DAB produced no tumors in six dogs in 63 months. Only AAT caused hepatic and gall-bladder tumors, but both caused urinary-bladder tumors.

Schmähl (1954) fed rats 5-10 mg/day of 2-hydroxy-4-dimethyl-aminoazobenzene until they had received 2.5 g. Weight gain was normal and no tumors developed. The livers had a normal appearance.

Brown et al (1954) prepared some analogs of 4-dimethylamino-azobenzene (DAB) in which the non-amino benzene ring had been replaced by a pyridine, pyridine-N-oxide, or thiazole ring and tested them against DAB in rats at 0.06% of their low-protein, low-riboflavin diet. Table 98 contains the tumor incidence, survivability, hepatic histology, and 3'-methyl DAB comparison data. The latter was from a follow-up study on PO4; because of the latter's toxicity, one day each week for the first

TABLE 98.

TUMOR INCIDENCE* AMONG THE VARIOUS GROUPS OF RATS RECEIVING HETEROCYCLIC ANALOGS OF DAB

Compound	Code	4 mo.	6 mo.	8 mo.	10 mo.	12 mo.
Pyridine-2-azo-*p*-dimethylaniline	P-2	0/2	0/2	0/2	0/2	0/2
Pyridine-3-azo-*p*-dimethylaniline	P-3	0/2	0/2	0/2	0/2	0/2
Pyridine-4-azo-*p*-dimethylaniline	P-4	0/2	2/2	4/4	no survivors	
Thiazole-2-azo-*p*-dimethylaniline	T-2	0/2	0/2	0/2	0/2	0/2
Pyridine-1-oxide-2-azo-*p*-dimethylaniline	PO2	0/2	0/2	0/2	0/2	0/2
Pyridine-1-oxide-4-azo-*p*-dimethylaniline	PO4	5/5	no survivors			
p-Dimethylaminoazobenzene	DAB	1/3	2/3	2/2	2/2	no survivors

* Tumor incidence = number of rats with hepatic tumors/number of rats sacrificed.

SUMMARY OF HISTOLOGICAL DATA OBTAINED FROM THE LIVERS OF RATS RECEIVING HETEROCYCLIC ANALOGS OF DAB

Code	4 months	6 months	8 months
P-2	Diffuse fatty changes to normal	Moderate fatty changes	Moderate fatty changes
P-3	Moderate fatty changes, less than P-2	Normal	Slight irregularity of lobular pattern, some large or double nuclei
P-4	Slight fatty changes	Nodular tumors; two kinds of neoplasm-hepatoma and papillary adenocarcinoma arising from bile ducts	Large nodules of necrotic tumor of liver cell type
T-2	Marked fatty changes	Normal	Normal
PO2	Normal	Moderate fatty changes	Normal
PO4	Liver entirely replaced by papillary-type tumor, fibrous tissue reaction, acute inflammatory necrosis	No survivors	
DAB	One animal with multiple tumor nodules, fibrous tissue reaction, inflammation, fatty changes; two animals, livers normal	Two animals with tumor masses of liver cell type surrounded by slight fatty changes; one animal liver normal	No survivors
Control	Normal	Normal	Normal

RESPONSES OF RATS RECEIVING PO4 AND 3'-METHYL-DAB

	PO4	3'-Me-DAB
Survival (4 months)	2/5	4/5
Average body weight of survivors	145 gm.	230 gm.
Weight change (3 months)	— 19 gm.	32 gm.
Liver weight as per cent of body weight	15 per cent	9 per cent
Food consumption (gm/day)	7	12
Tumor formation (4 months)	4/5 had massive tumor formation; 1/5 had nodulation and small tumors	3/5 had extensive nodulation to definite tumors; 1/5 had slight nodulation; 1/5 normal

six weeks only the base diet was given (also to the 3'-methyl DAB control).

Sugiura et al (1954) compared the hepatocarcinogenicity of some compounds similar to DAB, in rats; four of these were new, the 4th, 7th, 9th, and 10th compounds in Table 99. Dye intake was initially about 6 mg/day; this fell to 3 mg/day in those rats with liver damage

TABLE 99.—*Incidence of hepatic tumors in rats fed various azo compounds*

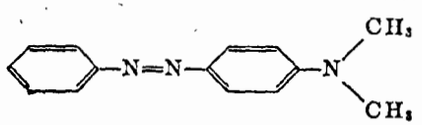
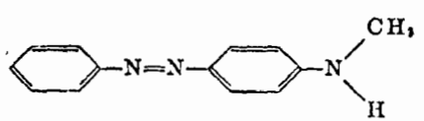
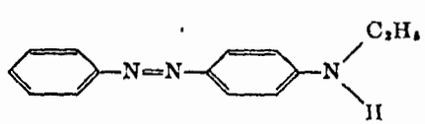
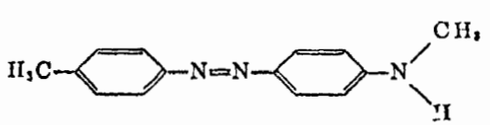
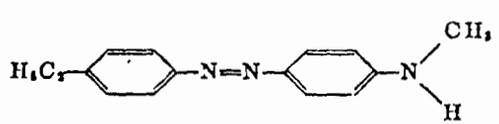
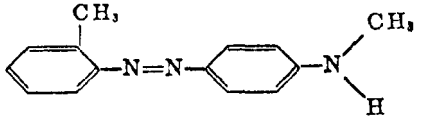
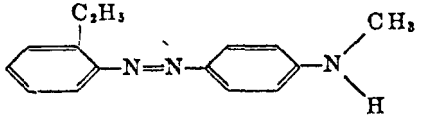
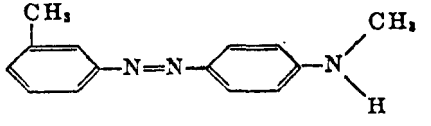
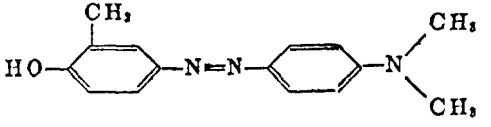
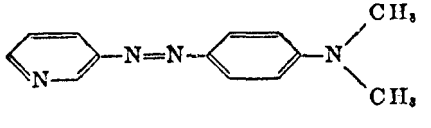
Compound fed	Formula	No. of animals	Percent in diet	No. of days fed	Liver findings at autopsy*					Incidence of liver cancer (*percent*)
					−	±	+	++	+++	
N,N-Dimethyl-*p*-aminoazobenzene		15	0.060	75–250	0	1	3	5	6	93
N-Methyl-*p*-aminoazobenzene		14	0.056	132–250	0	1	5	3	5	93
N-Ethyl-*p*-aminoazobenzene		15	0.060	148–250	15	0	0	0	0	0
N-Methyl-4′-methyl-*p*-aminoazobenzene		15	0.060	100–250	8	4	3	0	0	20
N-Methyl-4′-ethyl-*p*-aminoazobenzene		15	0.064	104–223	0	0	7	7	1	100

TABLE 99. Continued

N-Methyl-2′-methyl-*p*-aminoazobenzene.		15	0. 060	98–250	10	1	3	0	1	27
N-Methyl-2′-ethyl-*p*-aminoazobenzene.		15	0. 064	74–250	15	0	0	0	0	0
N-methyl-3′-methyl-*p*-aminoazobenzene.		15	0. 060	141–225	0	0	6	6	3	100
N,N-Dimethyl-3′-methyl-4′-hydroxy-*p*-aminoazobenzene		15	0. 068	107–250	15	0	0	0	0	0
3′-(4-Dimethylaminophenyl) azopyridine.		15	0. 060	109–250	4	3	2	4	2	33

*—indicates smooth, practically normal liver; ± indicates nodular cirrhosis with adenomatous hyperplasia; + indicates distinct areas of cholangioma or hepatoma; ++ indicates extensive liver cancer without metastasis; +++ indicates extensive liver cancer with metastasis.

and tumors who ate less food.

Brown et al (1954, pp. 715-717) followed up their earlier 1954 publication (see above) dealing with the carcinogenicity of pyridine- and pyridine-N-oxide azodimethylanilines. Their results are given in Tables 100 and 101.

TABLE 100.

TUMOR INCIDENCES OF RATS FED VARIOUS PYRIDINE ANALOGS OF DAB

Compound	Code	Level in diet (per cent)	Tumor incidence* 1 mo.	2 mo.	3 mo.	4 mo.	5 mo.	6 mo.	8 mo.	10 mo.
4-Methylpyridine-2-azo-*p*-dimethyl-aniline	4-Me-P2	0.06				0/2		0/2	0/2	0/2
6-Methylpyridine-2-azo-*p*-dimethyl-aniline	6-Me-P2	"				0/2		0/2	0/2	0/2
4-Methylpyridine-1-oxide-2-azo-*p*-dimethylaniline	4-Me-PO2	"				0/2		0/2	0/2	0/2
6-Methylpyridine-1-oxide-2-azo-*p*-dimethylaniline	6-Me-PO2	"				0/2		0/2	0/2	0/2
2-Methylpyridine-4-azo-*p*-dimethyl-aniline	2-Me-P4	"		0/2		0/2	2/3	2/2		
Pyridine-1-oxide-3-azo-*p*-dimethyl-aniline	PO3	"		0/2		1/2	0/2	3/3		
2-Methylpyridine-1-oxide-4-azo-*p*-dimethylaniline†	2-Me-PO4	"				7/7	no survivors			
3-Methylpyridine-1-oxide-4-azo-*p*-dimethylaniline	3-Me-PO4	"	0/1	0/2		0/2	2/2	2/2		
p-Dimethylaminoazo benzene	DAB	"		0/2		1/3		2/3	2/2	2/2
2-Methylpyridine-1-oxide-4-azo-*p*-dimethylaniline	2-Me-PO4	0.02	0/2‡	0/4‡						
2,6-Dimethylpyridine-1-oxide-4-azo-*p*-dimethylaniline	2,6-diMe-PO4	0.02–.03§		0/2‡	0/2‡	2/2		1/1		

* Tumor incidence is the number of rats with hepatic tumors/number of rats sacrificed.

† Diet level was reduced by intermittent use of carcinogen-free diet. The number of days on and off the carcinogenic diet are indicated by the following sequence where numbers in parentheses are days on basal diet with no carcinogen. 11-(8)-6-(1)-1-(2)-11-(2)-50-(4)-34.

‡ These rats, while having no tumors, did show advanced cirrhosis.

§ At the end of the first month the level was increased to 0.06 per cent.

TABLE 101.

RESPONSES OF RATS RECEIVING 2-ME-PO4 AND *m'*-METHYL-DAB*

	2-Me-PO4	*m'*-Me-DAB
Survival at 4 months	7/10	10/10
Av. body weight of survivors	167 gm.	290 gm.
Av. weight change at 4 months	9 8 gm.	152 gm.
Av. liver weight as per cent of body weight	27.6 per cent	5 5 per cent
Tumor formation at 4 months	7/10 had massive tumor formation; 3/10 died within 2 weeks of start	5/10 slight necrosis; 5/10 normal

* The carcinogens at the 0.06 per cent level were fed intermittently to both groups in identical manner. The number of days on and off the carcinogenic diet are indicated by the following sequence where numbers in parentheses are days on basal diet with no carcinogen. 11-(8)-6-(1)-1-(2)-11-(2)-50-(4)-34.

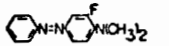
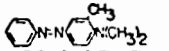
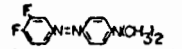
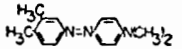
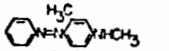
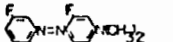
Badger et al (1954) tested a variety of azo compounds for carcinogenicity in rats fed a low protein, low riboflavin diet in amounts molar-equivalent to 0.06% for DAB. For DAB, 7/7 and 8/8 survivors had tumors, for 4'-methoxy DAB, 4/10 survivors had tumors, and for the following compounds there were no tumors (number in parentheses is % surviving): 2,2'-azonaphthalene (90), 1-phenylazonaphthalene (50), 2-phenylazonaphthalene (60), azobis(4-dimethylamino)benzene (80), 4-methoxyazobenzene (70), and azobis(4-methoxy)benzene (80).

Bonser et al (1954) gave mice s.c. injections of 3 mg of 1-(2-tolylazo)-2-naphthol (Oil Orange TX) twice a week for 50 weeks. Intestinal tumors appeared at 62 weeks.

Miller et al (1957) prepared a variety of DAB-related compounds and fed them to rats to determine the hepatocarcinogenicity against DAB as a control. The results are in Tables 102, 103, and 104. These tables also include the results of studies on possible metabolites and rearrangement products. The authors concluded that the 2- position of the amino-ring of DAB must not be substituted in order to retain hepatocarcinogenicity. In Table 105 is a listing of relative carcinogenicities of substituted DAB's.

TABLE 102.

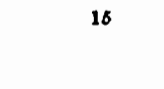
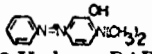
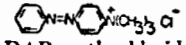
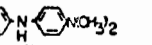
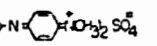
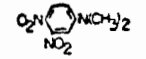
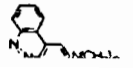
THE CARCINOGENICITIES OF CERTAIN FLUORO AND METHYL DERIVATIVES OF 4-DIMETHYLAMINOAZOBENZENE

Series	Group	Compound fed (2.40 millimoles/kg diet No. 1)	Time compound was fed (mo.)	No. of rats started	Incidence of liver tumors* (mo.) 3	4	5	6	7	8	10	11	Gross cirrhosis at end of feeding of compound
I	1	 4-Dimethylaminoazobenzene(DAB)	4	16		4/16		14/16					Mild-moderate
	2	 3-Fluoro-DAB	5	18			2/18		10/18				Mild
	3	 3-Methyl-DAB	8	11						0/11		2/11†	None
	4	 3',4'-Difluoro-DAB	3	15	12/13	13/13							Moderate-severe
	5	 3',4'-Dimethyl-DAB	6	12				0/12		2/12			None
II	6	DAB	4	16		6/15		14/15					Mild-moderate
	7	 2,6-Difluoro-DAB	7	14					0/14			0/14	None
III	8	DAB	4	16		3/16		11/16					Mild-moderate
	9	 2-Methyl-MAB‡	8	16						0/16	1/16		None
	10	 2,6,3',5'-Tetrafluoro-DAB	6	12				0/12		0/12			None
	11	 2,6,2',4',6'-Pentafluoro-DAB	4	6		0/6					0/6		None
IV	12	DAB	4	16		5/16		13/16					Mild-moderate
	13	 2,5,2',5'-Tetrafluoro-DAB	5	10			0/10		6/10				Mild

* Number of rats with tumors/number of rats alive at end of dye feeding. † Benign hepatomas. ‡ MAB = 4-monomethylaminoazobenzene.

TABLE 103.

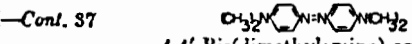
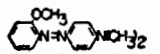
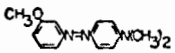
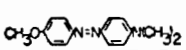
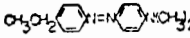
THE CARCINOGENICITIES OF VARIOUS REARRANGEMENT PRODUCTS AND OTHER DERIVATIVES OF 4-DIMETHYLAMINOAZOBENZENE

Series	Group	Compound fed (2.67 millimoles/kg diet No. 2)	Time compound was fed (mo.)	No. of rats started	Incidence of liver tumors (mo.) 4	6	9	10	11	12	Gross cirrhosis at end of feeding of compound
V	14	DAB	4	14	4/14	12/14					Mild-moderate
	15	 2-Acetylamino-5-dimethylamino-diphenylamine	8	15					0/13		None
	16	 Benzo(c)cinnoline	8	11					0/11		None
	17	 3-Hydroxy-DAB	8	6					0/6		None
VI	18	DAB	4	16	15/16	15/16					Severe
	19	2-Nitro-5-dimethylamino-diphenylamine	8	15					0/13		None
	20	 DAB methochloride	8	16					0/14		None
	21	3-Hydroxy-DAB (cf. group 17)	8	6					0/6		None
VII	22	DAB	4	15	12/15	14/15					Moderate
	23	 4-Amino-4'-dimethylamino-diphenylamine	8	15				0/14			None
	24	 4-Imino-4'-dimethylaminodiphenyl-imine sulfate	8	15				0/15			None
VIII	25	DAB	4	12	6/12	12/12					Moderate
	26	 3,4-Dinitrodimethylaniline	12	10						0/12	None
IX	27	DAB	4	15	10/15	12/15					Moderate
	28		7	6			0/6				None

1514

TABLE 104

THE CARCINOGENICITIES OF VARIOUS DERIVATIVES OF 4-DIMETHYLAMINOAZOBENZENE

Series	Group	Compound(s) fed	Millimoles/kg diet*	Time compound was fed (mo.)	No. of rats started	Incidence of liver tumors† (mo.) 3	4	6	8	9	17	Gross cirrhosis at end of feeding of compound
XII—*Cont.*	37	 4,4′-Bis(dimethylamino)-azobenzene‖	2.67	15	16						0/13	None
XIII	38	DAB	2.40**	4	16	1/16	7/16	10/16				Moderate
	39	 2′-Methoxy-DAB	2.40**	6	16		0/16	5/16	8/16			Mild
	40	 3′-Methoxy-DAB	2.40**	3	16	8/9	9/9					Severe
	41	 4′-Methoxy-DAB	2.40**	6	16		0/16	5/16	12/16			Mild
XIV	42	DAB	2.00	3	16	0/16		4/16				None-mild
	43	3′-Methyl-DAB	2.00	3	16	6/16		16/16				Moderate
	44	3′-Methoxy-DAB	2.00	3	16	8/16		16/16				Moderate
XV	45	DAB	2.14††	3	14	1/14	5/14					Mild
	46	 4′Ethyl-DAB	2.14††	3	14	6/13	12/13					Severe

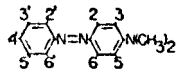
‖ Compound may be poorly absorbed, since it was readily detected in the feces.

** Fed 2.67 millimoles of dye/kg diet for 2 weeks, then on dye-free diet for 9 days and returned to level of dye listed above for remaining time because of toxicity of 3′-methoxy dye.

†† Fed 2.67 millimoles of dye/kg diet for 6 weeks and then level of dye listed for remaining time because of toxicity of 4′-ethyl dye.

TABLE 105.

THE CARCINOGENICITIES OF VARIOUS RING-SUBSTITUTED DERIVATIVES OF 4-DIMETHYLAMINOAZOBENZENE*



	Relative activities (unsubstituted dye = 0)								
Position	F-	CH_3-	CH_3CH_2-	Cl-	Br-	NO_2-	CF_3-	HO-	CH_3O-
4′	10–12	<1†	10†	1–2		0‡	0	0	3
3′	10–12	10–12†		5–6	0§	5‡	0	0	10–12
2′	7	2–3†	0#	2		3	0	0	2
2	>10	0†						0	
3	4	<1†						0	
2′, 4′	>10	0							
2′, 5′	>10	0		0					
3′, 4′	>10	<1							
3′, 5′	>10	0							
2, 6	0								
2′, 4′, 6′	>10			0	0‡				
2, 6, 3′, 5′	0								
2, 5, 2′, 5′	4								
2, 6, 2′, 4′, 6′	0								

* Data from Refs. 22–24, 26, and present paper except where noted.

† The N-monomethyl dyes have similar activities; see Refs. 23, 35, 36 and present paper.

‡ Poorly absorbed.

§ See Ref. 16.

As N-monomethyl derivative (36).

Nieper and Druckrey (1957) reported that Janus Green did not cause tumors in rats either from 670 daily oral doses of 20 mg in their food, or from 66 fortnightly s.c. injections of 0.5 mg. After cessation of either treatment the rats were allowed to live out their normal lifespan.

Masusaki (1958) tested the effectiveness of some azo compounds on the in vitro inhibition of Ehrlich cancer cells by i.p. injecting the so-treated cells into mice and measuring their longevity. In decreasing order of effectiveness the compounds were: 6-(2'-hydroxy-3',5'-dibromophenylazo)-4-hexylresorcinol, the 3',5'-dichloro derivative, the 5'-carboxy = the 5'-methyl = the 4',6'-dibromo-2'-carboxy = 6-(2'-hydroxy-3',5'-dibromophenylazo)-4-carboxyethylresorcinol = bis(2-hydroxy-3,5-dibromophenylazo)-L-tyrosine, 2,2'-dihydroxyazobenzene = 1-(2'-hydroxy-3',5'-dibromophenylazo)-2-naphthol-3,6-disulfonic acid = 6-(2'-hydroxy-

4'-sulfaminophenylazo)-4-hexylresorcinol (the last three had no effectiveness). The cell treatment was temperature and azo concentration dependent.

Rüttner and Brunner (1959) were unable to induce tumors in rats by fortnightly i.p. injection of 1 ml of a 2% solution of Trypan Blue (18-19 injections) or Evans Blue (12 injections). The animals were observed for 7-9 months after injections ceased, then sacrificed.

Mulay and O'Gara (1959) fed male and female rats 4-dimethylaminoazobenzene (DAB), 4'-dimethylaminophenylazo-1-naphthalene (DAN), or 4'-dimethylaminophenylazo-2-naphthalene (DA-2-N) with the results in Table 106. The DAB and protein 8 treatment was an 8% rather than the usual 12% protein diet. Except for DA-2-N the sex difference in tumor development was striking. Average time for tumor development from DAN was 20% longer in the females. This figure was considerably higher from DA-2-N treatment.

Table 106

Incidence of Liver Tumors in Osborne-Mendel Rats Fed Carcinogenic Dyes.

Treatment				Tumors induced			
Carcinogen	%	Days fed	Induction period (days)	♂ Incidence	♂ %	♀ Incidence	♀ %
DAN	.075	300	270	43/57	75	8/48	17
	.15	"	"			1/9	11
	.3	"	"			3/5	60
	.6	"	"	2/4	50		
DA-2-N	.075	230	80	39/40	98	24/24	100
DAB	.06	280	150	56/66	85	6/31	19
DAB and protein 8	.06	"	"			2/52	4

Gelstein (1961) found hepatic tumors in the first and second generation offspring of mice dosed with o-aminoazotoluene. Of those young born during the dosage period and kept with their dams for one

month, 65% developed tumors. Incidence of tumors in second generation mice was nearly four times higher than that seen in controls.

Brown and Hamdan (1961) fed a variety of 4'-alkyl substituted 4-dimethylaminoazobenzenes (DAB) to rats at 0.06% of the low-protein, low-riboflavin diet and examined them at two-month intervals for hepatic tumors. DAB gave a 90% incidence at six months. The n-Bu DAB gave a 43% incidence in 12 months (0% at six), the t-Bu DAB 33% at 12 months (0% at six), the EtDAB and i-PrDAB 100% at four months (toxic), the n-PrDAB 78% at six months, and MeDAB, iso-BuDAB, and sec-BuDAB 0% at 10, 12 and 12 months; the phenyl DAB gave 0% at six months. At the 0.03% dietary level the iso-PrDAB and EtDAB were 50% and 267% more active than 0.03% DAB at six months.

Arcos and Griffith (1961) found that a seven-month feeding of 0.04% 2-methylDAB or 0.02% 3'-methyl DAB to rats gave 0/22 and 1/23 incidences of hepatic tumors, respectively, but a combination of these two dietary levels gave a 5/20 incidence at seven months. While 0.035% of 3'-methyl DAB gave incidences of 14/24 at four months and 24/24 at six months, adding 0.035% of 2-methyl DAB to this diet gave incidences of 10/24 at four and 21/23 at six months. Feeding rats 0.06% 2-methyl DAB for three months prior to five months of 0.054% DAB had no effect on tumor incidence.

Takayama (1961) studied the synergism of DAB feeding and skin painting with 3-methylcholanthrene (MC) or 4-nitroquinoline N-oxide (NQNO), none administered in amounts individually capable of inducing tumors. Three months of 0.5 g daily oral doses in food of DAB failed to generate hepatic tumors, but the same treatment followed by six months

on a no-DAB diet and twice-weekly painting with MC or NQNO produced one hepatic tumor (of eight surviving rats on day 420) from MC, and, from the NQNO, four tumors in 23 rats dying between days 160-420, and two tumors in three rats still alive on day 420. Treatment of six months painting by MC or NQNO followed by three months of DAB feeding produced no tumors in the liver.

Miller and Miller (1961) tested the tumor inducing abilities in rats, against DAB controls, of some hydroxy and methoxy substituted 4-amino-, methylamino-, and dimethylaminoazobenzenes. Dosage was equivalent on a molar basis to 0.06% DAB. Table 107 contains the results.

TABLE 107.

THE INCIDENCES OF TUMORS IN RATS FED THE 2-HYDROXY (HO-) OR 2- OR 3-METHOXY (MeO-) DERIVATIVES OF 4-AMINOAZOBENZENE (AB), 4-MONOMETHYLAMINOAZOBENZENE (MAB), OR 4-DIMETHYLAMINOAZOBENZENE (DAB)

Exp. no.	Compound*	Av. initial wt. (gm.)	Av. wt. gain at 3 wk. (gm.)	Time cpd. fed (mo.)	Sex	Survival at 4 mo.†	No. rats with malignant tumors of the:									Negative survivors (11 mo.)
							Liver		Ear duct			Small intestine	Mammary gland		Skin	
							(6 mo.)	(11 mo.)	(6 mo.)	(9 mo.)	(11 mo.)	(11 mo.)	(6 mo.)	(9 mo.)		
1	3-MeO-AB	237	20	8	M	9/16	0	1	1	6	7	3	0	0	2‡	0
	3-MeO-MAB	231	12	8	M	12/16	0	0	2	9	10	1	0	0	2§	0
	3-MeO-DAB	210	21	8	M	13/16	0	0	0	5	7	0	0	0	1#	5
	2-MeO-AB	240	33	8	M	5/16	0	0	0	0	0	0	0	0	0	5
	2-MeO-MAB	242	21	8	M	9/16	0	0	0	0	0	0	0	0	0	7
	2-MeO-DAB	234	16	8	M	14/16	0	0	0	0	0	0	0	0	0	11
	2-HO-DAB	235	27	8	M	15/16	0	0	0	0	0	0	0	0	0	9
	DAB	232	2	5	M	15/16	10	13	0	0	0	0	0	0	0	2
2**	3-MeO-AB	235	25	8	M	11/13	0	0	0	6	7	3	0	0	0	1
3	3-MeO-AB	201	21	8	F	17/20	0	0††	1	7		0††	2	3	0	1††
	AB	202	18	8	F	15/15	0	0††	0	0		0††	1	1	0	7††
	None	203	31	8	F	14/15	0	0††	0	0		0††	1	1	0	10††
4	DAB	242	77	5	M	16/18	5	12††	0	0		0††	0	0	0	3††
	2-HO-MAB	209	86	5	M	9/10	0	0††	0	0		0††	0	0	0	5††

* 2.67 mmoles/kg of diet.

† No. of rats alive at 4 mo/no. started on diet.

‡ These squamous-cell carcinomas, one on the lip and one on the skin of the back, were found at 11 mo.

§ One basal-cell carcinoma was found at 8 mo., and a squamous-cell carcinoma was found at 9 mo. Both were located on the skin of the back.

A basal-cell carcinoma on the skin of the back was first observed at 11 mo.

** Three groups of four, four, and five rats were fed the basal diet described in "Methods," the basal diet plus 20 mg. of 2-methyl-1, 4-naphthoquinone/kg, or the basal diet in which crude casein was substituted for vitamin-low casein for 5 weeks. This experiment was set up as a result of the loss of four of sixteen rats fed 3-methoxy-AB during the first 3 weeks of the first experiment, the objective was to find more favorable conditions for administration of the compound. Since there appeared to be no differences in the rats fed these three diets by 5 weeks, the animals from all three groups were fed the basal diet described in the "Methods" for the remainder of the experimental period.

†† These data were obtained at 9 mo., the time at which the surviving rats of Exp. 3 were killed.

Grice et al (1961) fed rats for 65 weeks on a diet containing 0.3, 1.0 or 3.0% of Ponceau 3R, a commercial dye consisting of a mixture of many azo components. Hepatic tumors were present in 2/24 rats at the 1% level, and 7/23 at the 3% level.

Terracini and Della Porta (1961) fed hamsters DAB, thrice-weekly stomach tube 10 mg doses for three weeks, 5 mg for the next seven weeks, and 10 mg for the last 32 weeks--survivors receiving 1.155 g. Other animals received 3'-methyl DAB as 0.064% of their diet for 27 weeks, then 0.1% for 11 weeks--survivors receiving about 1.4 g. There were no hepatic tumors seen by 48 weeks after cessation of treatment with DAB. One hepatic tumor was found 23 weeks after cessation of treatment with 3'-methyl DAB. Fifteen animals of each sex had been used for each azo compound.

Brown et al (1961) prepared all of the 4-dimethylaminoazo-quinolines (Q) and quinoline N-oxides (QO). Initially all of these were fed to rats, except the 2-quinoline isomer, at 0.03% of a low-protein, low-riboflavine diet along with no-dye, 0.03% and 0.06% DAB controls. The more tumor-active compounds were then given at the 0.01% level. Results are given in Tables 108 and 109. The number following Q or QO refers to the position of the quinoline or quinoline N-oxide which bears the azo linkage.

Table 108

Carcinogenicities of quinoline dyes in relation to DAB*

Code	Incidence of liver tumors†	
	4 Months	6 Months
Control (no dye)	0/10	0/10
DAB‡ (0.06%)	7/10	5/10
DAB	0/10	5/10
3′-Me-DAB§	5/11	10/11
QO2‖	0/10	0/10
Q3¶	0/10	1/10
QO3	0/10	0/10
Q4	5/10	9/9
QO4	8/10	10/10
Q5	9/9	—
QO5	10/10	—
Q6	9/9	—
QO6	9/10	10/10
Q7	0/10	0/10
QO7	0/10	0/10
Q8	0/10	0/10
QO8	0/10	0/10

*Level in diet, 0.03 percent unless noted.
†Number of rats with tumors/number of rats in experiment.

Table 109

Comparison of the carcinogenicities of quinoline dyes*

Code	Incidence of liver tumors† (4 months)
Q5	8/9
QO5	10/10
Q6	10/10
QO6	5/10

*Level in diet, 0.01 percent.
†Number of rats with tumors/number of rats in experiment.

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Burkhard et al (1962) synthesized 2'-, 3'-, and 4'-methylthio DAB, also 4-methylthioazobenzene. These were fed as 0.06% of the diet (except 0.03% in the first two weeks for the relatively toxic 4'-methyl DAB) to rats. No hepato tumors appeared after 23 and 25 weeks of feeding the 2'-Me-S-DAB or 4-Me-S-azobenzene, respectively. After 16 weeks of feeding 3'-Me-S-DAB 16/19 rats had these tumors, and after 20 weeks of feeding 4'-Me-S-DAB 13/16 rats had tumors.

Weisburger and Weisburger (1963) reviewed the pharmacodynamics of carcinogenic azo compounds, pointing out that metabolic "activation" by the host animal seemed to be required, and interspecies differences in efficiency in doing this probably accounted for part of the relatively small number of species susceptible to azo compound carcinogenesis.

Brown (1963) fed rats 0.03% of 4-dimethylaminoazoisoquinoline-4, -5, and -7, also -isoquinoline-N-oxide-5. After four months there were: no hepatic tumors from -4 and -7, 7/7 from -5, and 1/10 from -N-oxide-5 (all 10 died in one month). After six months there was a 100% incidence of tumors in -4 and -7 with toxicity showing up from the latter. Retesting of -5 and -N-oxide-5 at 0.01% level produced 0/7 and 8/8 tumors, respectively, after four months, and 1/7 tumors for -5 after six months. In

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Schmähl et al (1963) found that 233 daily doses of 33 mg/kg of DAB, or of 3 mg/kg of diethylnitrosamine (DENA) were sufficient to induce hepatocarcinogenesis in rats. However, on giving these compounds together in the diet at the mentioned daily dose, the time to generate the tumors was reduced to 153 days. Although animals fed only DENA gained much more weight than those fed only DAB in the diet, those on the combined diet had a weight gain-time curve almost superposable on that

of DAB alone.

Silva and Brandt (1964) demonstrated that 1,2'-azonaphthalene was effective against transplanted Walker 256 carcinosarcoma in rats. Both i.p. and i.v. injections worked, but best results came from using both methods of introduction.

Manchon (1965) reviewed data relevant to food dyes.

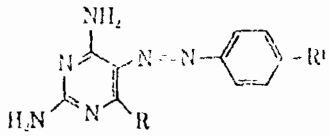
Huggins and Pataki (1965) investigated the ability of pre-administered azo compounds to protect against tumor genesis by 7,12-dimethylbenz(a)anthracene. A 20 μg dose of Sudan III was most effective.

Druckrey et al (1965) determined that weekly s.c. injections into rats of 50 mg/kg of azoethane would produce a variety of tumors in 37 weeks. Doubling the dose only reduced the induction time to 33 weeks.

Hampshire et al (1965) prepared some 2,4-diamino-5-arylazo-pyrimidines and determined their toxicity to rats and mice, antitumor activity against Murphy-Sturm lymphosarcoma in rats, and inhibition of rat hepatic folic acid reductase. The compounds used are given in Table 112 (except for III), and the test data in Table 113. The mouse toxicity LD-50's are for a single i.p. dose and a 21-day observation period. The rat toxicity was determined during the anti-tumor testing which consisted of five daily i.p. injections starting five days after tumor implantation. There was no correlation between the enzyme inhibition and the tumor repression.

TABLE 112.

2,4-DIAMINO-6-SUBSTITUTED 5-ARYLAZOPYRIMIDINES



No.	R	R'
II	NH_2	H
III	Cl	$COOC_2H_5$
IV	Cl	$CONHCH(CO_2H)(CH_2)_2CO_2H$
V	NH_2	$COOC_2H_5$
VI	NH_2	SO_2NH_2
VII	NH_2	$CONHCH(CO_2H)(CH_2)_2CO_2H$
VIII	NH_2	COONa
IX	NEt_2	$COOC_2H_5$
X	NEt_2	$CONHCH(CO_2H)(CH_2)_2CO_2H$
XI	NH_2	NEt_2
XII	NH_2	$N(CH_2CH_2Cl)_2$
XIII	NH_2	$N(CH_2CH_2Br)_2$
XIV	NH_2	$N(Et)CH_2CH_2Cl$
XV	N⟨piperazine⟩NCH$_2$CH$_2$OH	COOEt
XVI	N⟨piperazine⟩NCH$_2$CH$_2$Cl·HCl·2H$_2$O	COOEt

TABLE 113.

ANTITUMOR AND FOLIC ACID REDUCTASE DATA

No.	Folic acid reductase inhibition[a] $(I_1/[S])_{50}$	Mouse toxicity,[b] mg./kg.	Rat toxicity,[c] mg./kg. q.d. 5–9	Antitumor activity[d]: Dose, mg./kg. q.d. 5–9	Antitumor activity[d]: % body wt. change[e]	Antitumor activity[d]: T/C[f]
II	0.001	170	80	30	−1	0.6
IV	4.0	>1000				
V	0.011	>1000	>400	400	+1	0.3
VI	1.1	500				
VII	1.8	>1000	>100	100	+18	1.05
VIII	1.6	80	220	200	−13	0.2[illegible]
				100	+16	0.9
IX	3.75	400	>200	200	+20	0.[illegible]
X	0.125	>1000	>200	200	+18	[illegible]
XI	0.1	240				
XII	0.17	290				
XIII	0.15					
XIV	0.125	170				
XV	0.[illegible]	>1200	>400	[illegible]	+10	0.85
XVI	1.4	180	140	0	−13	0.13
IAP[h]	5.0	600				

[a] The [illegible] concentration of inhibitor required for 50% inhibition of enzyme activity [illegible] Adenocarcinoma 755 [illegible] [illegible] conjugation. [illegible] solubility of the compound [illegible] IAP is 2,4-diamino [illegible]

Fare (1966) painted the dorsal skin of rats (1 ml) and mice (0.2 ml) with a 0.2% acetone solution of some 4-amino azobenzenes twice a week. Results are in Table 114. The compounds were chromatographically pure.

TABLE 114.

RESULTS OF PAINTING SKIN OF RATS AND MICE WITH AZO DYES

Species	Sex	Dye	Mean time 1st lesion noted (wk.)	Mean length of treatment (wk.)	Tumor incidence		Total yield of skin tumors in group, postmortem[a]						
					Ear duct	Skin	Epidermoid cyst	Keratoacanthoma	Squamous carcinoma	Basal carcinoma	Anaplastic carcinoma	Squamous papilloma	Miscellaneous
Rat	M	None (Control)		131	0/6	0/6							
Rat	M	AAB[b]	97	123	0/6	6/6	5		4	8	2	1	3
Rat	M	MAB	44	58	0/6	6/6	16	2	7	18	9	2	7
Rat	M	DAB	73	90	0/6	6/6	2		3	11	4		3
Rat	M	3-MeO-AAB	76	93	1/6	6/6	3	1	3	5	5	2	5
Rat	M	3-MeO-MAB	28	41	2/6	6/6	27	2	12	28	13	2	6
Rat	M	3-MeO-DAB[c]	47	62	3/10	10/10	2	2	10	16	1	1	4
Mouse	F	3-MeO-DAB		30	0/140	0/140							
Mouse	M	3-MeO-DAB		62	0/140	0/140							

[a] Tumor types are described more fully in the text.

[b] Abbreviations: AAB, aminoazobenzene; MAB, monomethylaminoazobenzene; DAB, dimethylaminoazobenzene; MeO-, methoxy group.

[c] Data from Fare and Orr (4). The numbers of tumors produced are not comparable with those in the other treatment groups since in this particular case not all tumors were examined histologically.

Kanekar and Panse (1966) force fed rats five times a week with 0.4 ml of a 1% peanut oil solution of technical grade, or 0.4 ml of a 1% suspension in normal saline of purified 2',3-dimethoxy-4-aminoazobenzene. A tumor appeared in the skin at the external auditory canal opening after 156 days of dosing with the technical grade, and 174 days of the purified compound. Other rats survived 242 and 280 days, respectively, before developing this (and no other) type of carcinoma. These carcinomas were not transplantable.

Brown and Hamdan (1966) prepared and tested in rats some more nitrogen-heterocycle/azo/dialkylaminobenzene compounds. The results are

in Table 115. Except for the last three entries in the table, PO4 is an abbreviation for 4-[[p-(dimethylamino)phenyl]azo]-pyridine,1-oxide; using this nomenclature the entry above PO4 itself, e.g., should be written 2,3'DiMePO4. In the last three entries the dimethyl of (dimethylamino) has been replaced by the indicated alkyls.

Table 115. Rat Hepatocarcinogenesis from Alkylaminophenylazopyridine-N-oxides

Compound code	Percent in diet	Tumor incidence (months)				
		2	4	5	6	12
Control		0/10	0/10	0/10	0/10	0/10
DAB	0.06		7/10		9/10	
DAB	.03		0/10		5/10	
2'MePO4	.03		7/7			
2',6'DiMePO4	.03		4/4			
2'MePO4	.02		10/10			
2',6'DiMePO4	.02		10/10			
2'MePO4	.01		10/10			
2',6'DiMePO4	.01		9/10			
2,2'DiMePO4	.03		7/10		10/10	
2',3DiMePO4	.03		8/8			
PO4	.03	8/10	10/10			
MeEtPO4	.03		8/8			
DiEtPO4	.03	3/10				10/10
DiPrPO4	.03		0/10		0/10	0/10

Poirier et al (1967) prepared N-benzoyloxy-N-methyl-4-aminoazobenzene and tested its reactivity with various biochemicals, also its ability to generate carcinomas in rats after s.c. injection. Their results are in Tables 116, 117, 118, 119, and 120. Some closely related azobenzenes were tested for comparison.

TABLE 116.

Carcinogenic Activities of N-Benzoyloxy-N-methyl-4-aminoazobenzene and Related Compounds Administered by Repeated s.c. Injections in Rats

Each rat was injected s.c. in the right hind leg twice weekly with 0.2 ml of trioctanoin in which the test compound had been dissolved or suspended without heat immediately prior to injection.

Compound	Dose	No. of rats and sex	Average weight gain 1st 8 weeks (gm)	Cumulative No. of rats with sarcomas a. injection site				No. of rats with other tumors	Negative survivors[a]
				6 mo.	9 mo.	12 mo.	14-20 mo.		
Experiment 1									
N-Benzoyloxy-MAB[b]	24 × 3.9 mg	10 M	62	2	6	9	9		0
		10 F	48	2	5	10	10		0
MAB	24 × 2.5 mg	10 M	54	0	0	0	0	2, carcinomas of liver 1, carcinoma of carduct gland	8
		10 F	66	0	0	0	0	1, adenocarcinoma of mammary gland	9
None (vehicle only)	24 × 0.2 ml	10 M	69	0	0	0	0	1, sarcoma (distant from injection site)	9
		10 F	42	0	0	0	1	4, mammary fibroadenoma	9
Experiment 2									
N-Benzoyloxy-MAB	24 × 3.9 mg	20 M	159	9	20	20	20		0
MAB	24 × 2.5 mg	20 M	158	0	0	0	0	1, fibroma (distant from injection site)	15
N-Benzoyl-MAB	24 × 3.7 mg	20 M	162	0	0	0	0		19
N-Hydroxy-AB	24 × 2.5 mg	20 M	167	0	0	0	0		20
AB	24 × 2.3 mg	20 M	157	0	0	0	0		19
DAB-*N*-oxide	24 × 2.9 mg	20 M	166	0	0	0	1	1, adenocarcinoma of small intestine	17
DAB	24 × 2.7 mg	20 M	156	0	0	0	0		18
None (vehicle only)	24 × 0.2 ml	20 M	164	0	0	0	0	1, papilloma of skin	15

[a] Rats killed tumor-free at the termination of Experiments 1 and 2 at 20 and 14 months, respectively.

[b] The abbreviations used are: AB, 4-aminoazobenzene; MAB, *N*-methyl-4-aminoazobenzene; DAB, *N*,*N*-dimethyl-4-aminoazobenzene.

TABLE 117

Survival of Rats and Occurrence of Tumors after i.p. Injections of N-Methyl-4-aminoazobenzene (MAB) or Its N-Benzoyloxy Derivative into Neonatal Rats

Compound	Total dose (mg)	No. injected	No. alive at 23 days	No weaned and sex	No. of survivors at				No. of rats with gross tumors by 19 mo.
					7 mo.	10 mo.	13 mo.	19 mo.	
Experiment 1[a]									
N-Benzoyloxy-MAB	1.00	46	11	7 M	6	4	4	4	1, bilateral renal carcinomas (7 mo.) 1, multiple papillomas (urinary bladder) (19 mo.)
				4 F	4	4	3	1	1, bilateral renal carcinomas (12 mo.) 1, mammary gland carcinoma (19 mo.)
MAB	0.65	47	30	8 M[b]	8	8	8	7	0
				8 F[b]	8	8	8	7	2, mammary gland adenomas (19 mo.) 1, carcinoma *in situ* (skin) (19 mo.)
Corn oil only		33	25	8 M[b]	8	8	8	5	0
				8 F[b]	8	8	8	4	1, pulmonary adenoma (17 mo.)
Experiment 2[c]									
N-Benzoyloxy-MAB	0.48	162	98	56 M	55	48	28	8	3, pancreatic adenomas (13–15 mo.) 2, sarcomas (injection site) (13 and 14 mo.) 1, renal carcinoma (7 mo.) 1, basal cell carcinoma of lip (19 mo.) 1, cholangioma (19 mo.)
				42 F	38	32	25	9	2, mammary gland adenomas (19 mo.) 1, carcinoma of ear-duct gland (13 mo.) 1, cholangioma (19 mo.) 1, leiomyoma (small intestine) (19 mo.)
MAB	0.30	101	73	40 M	40	39	34	24	2, cutaneous papillomas (19 mo.) 1, malignant lymphoma (14 mo.)
				33 F	33	33	32	23	6, mammary gland adenomas (17–19 mo.) 1, mammary gland carcinoma (15 mo.) 1, cholangioma (17 mo.) 1, sarcoma (foot) (17 mo.)
Trioctanoin only		116	71	32 M	32	32	32	21	1, cutaneous papilloma (19 mo.)
				39 F	39	38	38	35	1, mammary gland carcinoma (8 mo.) 7, mammary gland fibroadenomas (15–19 mo.)

[a] Each rat was injected i.p. with 0.05 ml of sterile corn oil alone or containing 0.2 mg of *N*-benzoyloxy-MAB or 0.13 mg of MAB within 24 hr after birth and on each of the succeeding 2 days; on the 4th day the rats were injected with 0.1 ml of the same solutions.

[b] Because of the poor survival of the rats which received injections of *N*-benzoyloxy-MAB in this preliminary experiment, only 8 male and 8 female rats of the 30 and 25 rats injected with MAB or corn oil were kept at weaning.

[c] Each rat was injected i.p. with 0.05 ml of sterile trioctanoin alone or containing 0.16 mg of *N*-benzoyloxy-MAB or 0.10 mg of MAB within 24 hr after birth and on each of the succeeding 2 days.

TABLE 118.

The Reaction of N-Benzoyloxy-N-methyl-4-aminoazobenzene and Other Aminoazo Dyes with Bovine Serum Albumin[a]

	Protein-bound dye[b] (absorbance/50 mg protein)	
	Polar fraction	Nonpolar fraction
N-Benzoyloxy-*N*-methyl-4-aminoazobenzene	1.14 (520 mμ)	0.40 (507 mμ)
N-Methyl-4-aminoazobenzene	<0.01	0.01
N,*N*-Dimethyl-4-aminobenzene	<0.01	0.01
4-Aminoazobenzene	<0.01	0.02
N,*N*-Dimethyl-4-aminoazobenzene-*N*-oxide	0.01	0.02
N Hydroxy-4-aminoazobenzene	<0.01	<0.01
N-Hydroxy-*N*-acetyl-4-aminoazobenzene	<0.01	0.01

[a] Bovine serum albumin (125 mg) was incubated at pH 7 with 1.0 mg of *N*-benzoyloxy-*N*-methyl-4-aminoazobenzene or an equivalent amount of another dye for 4 hours at 37°C in a nitrogen atmosphere. The analytic procedure is described in the Materials and Methods section. The analyses for each of the polar and nonpolar fractions were corrected for blank values of 0.02 which were obtained when serum albumin was carried through the same procedure in the absence of any dye.

[b] The figures in parentheses are the wave lengths of maximum absorption for the dye fractions derived from *N*-benzoyloxy-*N*-methyl-4-aminoazobenzene; the corresponding fractions derived from the other dyes showed only low absorbances and for convenience were measured at the wave lengths shown.

TABLE 120.

The Reaction of N-Benzoyloxy-N-methyl-4-aminoazobenzene (N-Benzoyloxy-MAB) and Other Dyes with Various Nucleosides[a]

Experiment No.	Dye	Nucleoside	Radioactive product %[b] (based on limiting reactant)
1	*N*-Benzoyloxy-MAB	Guanosine-8-^{14}C	2.6
	MAB	Guanosine-8-^{14}C	0.0
	N-Hydroxy-4-aminoazobenzene	Guanosine-8-^{14}C	0.1
2	*N*-Benzoyloxy-MAB	Guanosine-8-^{14}C	3.3
	N-Benzoyloxy-MAB	Adenosine-8-^{14}C	0.0
	N-Benzoyloxy-MAB	Cytidine-2-^{14}C	0.0
	N-Benzoyloxy-MAB	Uridine-2-^{14}C	0.0
	N-Benzoyloxy-MAB	Thymidine-2-^{14}C	0.0
3	*N*-Benzoyloxy-MAB	Guanosine-8-^{14}C	5.0
	N-Benzoyloxy-MAB	Guanosine-8-^{3}H	5.1
4	*N*-Benzoyloxy-MAB	Guanosine-8-^{14}C	5.3
	N-Benzoyloxy-MAB	Guanosine-8-^{3}H	4.6

[a] The ingredients listed below in 1.4 ml of 28% ethanol were incubated under nitrogen at 37°C for the times indicated: Experiment 1, 0.02 μmole of guanosine, 0.5 μmole of dye, and 2 μmoles of sodium citrate buffer, pH 7, for 3.5 hours; Experiment 2, 0.08 μmole of nucleoside, 0.5 μmole of dye, and 2 μmoles of sodium citrate buffer, pH 7, for 3 hours; Experiments 3 and 4, 1.7 μmoles of guanosine, 1 μmole of dye, and 5 μmoles of sodium citrate buffer, pH 7, for 20 hours.

[b] The minimum reactions which could be detected were about 0.05 and 0.02%, for Experiments 1 and 2, respectively.

TABLE 119.

The Reaction of N-Benzoyloxy-N-methyl-4-aminoazobenzene (N-Benzoyloxy-MAB) with Amino Acids[a]

Dye	Amino acid	% reaction at 90 min[b]	Absorption maximum of water-soluble dye (mμ)
N-Benzoyloxy-MAB	Tryptophan	25	525
	Tyrosine	6	519
	Cysteine	6	522
	Methionine		
	Water-soluble dye	1.4[c]	518
	Total 3-methylmercapto-MAB released	3.6	
	Alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, or valine	<0.7	
MAB	Tryptophan, tyrosine, cysteine, or methionine	0.0	

[a] The amino acid (50 μmoles), 1.5 μmoles of dye, and 200 μmoles of sodium phosphate buffer, pH 7.0, in 2.5 ml of 20% ethanol were incubated in a nitrogen atmosphere at 37°C for 90 min. Except for the product formed from methionine, the extent of reaction was determined from the amount of water-soluble dye which remained after extraction with benzene-hexane.

[b] The % reaction was calculated on the assumption that the products had the same molar absorption coefficients as MAB. A blank equivalent to a reaction of 1.5% was subtracted from all values based on water-soluble dye when the amino acids were incubated with *N*-benzoyloxy-MAB. A blank of 0.0% was obtained when MAB was incubated and extracted as described.

[c] The sulfonium derivative formed from methionine gradually decomposes even at neutrality (see Chart 1). Therefore, the amount of 3-methylmercapto-MAB formed spontaneously plus that formed after the addition of alkali is a better estimate of the extent of reaction than the amount of water soluble dye.

Odashima and Hashimoto (1968) reported the following results of a 60-week feeding study on male rats. At a level of 0.08% 4-amino-azobenzene (AB) caused one peritoneal cavity tumor in 1/32 animals. At the 0.09% level 3-methoxy AB caused hepatocarcinoma in 21/23, malignant splenic hemangiopericytoma in 6/23, and squamous cell carcinoma of the ear duct in 2/23 animals. At the 0.1% level 3,4'-dimethoxy AB caused hepatocarcinoma in 24/24, squamous cell carcinoma of the ear ducts in 1/24, and tubulary adenocarcinoma of the small intestine in 2/24 animals. At the 0.09% level N-methyl AB and N,N-dimethyl AB caused only hepatic tumors, but did so more quickly than the other compounds.

Brown and Sanchorawala (1968) found the following incidences for hepatocarcinogenesis in rats at the indicated dietary levels: 0.06% DAB-70% at 4 and 90% at 6 months, 0.03% DAB-50% at 6 months, 0.03% 3'-methyl DAB-50% at 4 and 90% at 6 months, 0.03% N,N-dimethyl-p-(6-benzothiazolylazo)aniline-50% at 1 and 100% at 2 months, 0.03% N,N-dimethyl-p-(4-benzimidazolylazo)aniline-100% at 2 months, 0.03% N,N-dimethyl-p-(7-benzimidazolylazo)-aniline-100% at 3 months, 0.06% N,N-dimethyl-p-(4-benzothiazolylazo)aniline-0% at 6 months, and 0.03% N,N-dimethyl-p-(5-benzothiazolylazo)aniline-0% at 6 months.

Druckrey et al (1968) exposed 15-day pregnant rats for one hour to 4800 or 9600 ppm of azoethane, equivalent to 300 or 600 mg/kg (14 or 28% of the LD-50). At the lower dose 41/42 animals developed multiple neurogenic malignomas including 25 in the brain, 20 in the spinal cord, and 29 in the peripheral nerves. The corresponding numbers for the higher dosage group were 28/30, 16, 6, and 24. The

latter group also showed malformations of the paws. There may have been tumors in the dams, but the authors did not break out those tumors from azoethane (if any) and those from some related non-azo compounds also tested; in 32 dams there was a total of seven tumors.

Clayton et al (1968) fed rats a diet containing DAB derivatives at the molar equivalent of 0.06% DAB. All animals had hepato tumors at 9, 22, and 22 weeks in groups fed the 3'-methoxy, 3'-cyano, and 3'-acetylamino derivatives, respectively. All three caused severe cirrhosis in addition. No tumors resulted from 26-week feeding of the 2'-, 3'-, or 4'-carboxy, 2'- or 4'-methoxy, and 3',4'-dichloro; the 3',5'-dichloro produced one tumor in 12 rats at 26 weeks. In a follow up study for comparison with 3'-methyl DAB the three active compounds were fed for 51 days, then removed from the diet for eight weeks. At this time the percentage of survivors having tumors was: 3'-methyl 70, 3'-cyano 77, 3'-methoxy 85, and 3'-acetylamino 90.

Brown and Snider (1968) prepared an additional seven dimethyl-substituted derivatives of N,N,dimethyl-4-(4'-[pyridine-1-oxide]azo)aniline, designated PO4', in addition to the four reported on before. These were fed to rats at 0.03 and 0.06% of their low-protein, low-riboflavine diet along with 2'-methyl PO4', 3'-methyl PO4', and DAB, all for comparison. The results in generation of hepato tumors are given in Tables 121 and 122. The relative activities of the various compounds changed with the dietary level given.

Table 121

Tumor Incidences (0.03% level)

Compound code	Tumor Incidence (months)			
	4	5	6	8
DAB	0/10		5/10	
2'-MeP04'	10/10			
2,3-DiMeP04'	0/10	0/10	0/10	
2,3'-DiMeP04'	0/10	0/10	0/10	
2',5'-DiMeP04'	0/10	6/10		
2,5-DiMeP04'	0/10		2/10	
2,6-DiMeP04'	0/10		0/10	
3'-MeP04'	0/10	0/10	0/10	10/10[a]
3,3'-DiMeP04'	0/10	0/10	0/10	
3',5'-DiMeP04'	0/10	0/10	0/10	

Table 122

Tumor Incidences (0.06% level)

Compound code	Tumor incidence (months)			
	3	4	5	6
DAB		7/10		9/10
2,3-DiMeP04'	0/10	0/10	0/10	10/10
2,3'-DiMeP04'	0/10	10/10		
2',5'-DiMeP04'	0/10	10/10		
2,5-DiMeP04'	0/10	0/10	10/10	
2,6-DiMeP04'	0/10	0/10	0/10	10/10
3'-MeP04'	0/10	10/10		
3,3'-DiMeP04'	0/10	10/10		
3',5'-DiMeP04'	0/10	0/10	10/10	

Brown and Fisher (1969) prepared and tested for hepatocarcinogenicity in rats the following compounds at the 0.03% level: N,N-dimethyl-p-(3-,4-,5, or 7-indazolylazo)anilines--no tumors after 8 months; N,N-dimethyl-p-(6-indazolylazo)aniline--100% effective after 5 months;

Deleted due to copyright clearance difficulty.

N,N-dimethyl-p-(2-, 5-, or 6-quinoxalinylazo)anilines showed no tumors after 8 months, 100% tumors after 4 months, or 100% tumors after 2 months, respectively.

Bebavi et al (1970) prepared some new DAB derivatives, substituted in the 3',4' positions and compared them with DAB and 3'-methyl DAB for rat hepatocarcinogenicity after 50- or 365-day force feeding. Their results are in Table 124.

Table 124.
Carcinogenicities of the DAB's

A. 50 days of dye administration

DAB			Percentage of rats with hepatomas after start of dye administration[b]			
R-3'	R-4'	Dose[a]	4 mo.	8 mo.	12 mo.	Activity rank[c]
H—	Cl—	0.50	50 (50)[d]	75	100	4
Cl—	CH_3—	1.00	0 (10)	0 (10)[e]	0	0
Cl—	Cl—	1.00	0	0	0	0
H—	C_2H_5—	0.25	0 (15)	15 (15)	40 (10)	3
C_2H_5—	CH_3—	1.00	0 (60)	0 (10)	20 (10)	1
C_2H_5—	C_2H_5—	0.50	0	0	0	0
C_2H_5—	H—	0.50	0 (10)[f]	0	0	0
H—	H—	1.00	0	0	20	1
CH_3—	CH_3—	1.00	0	0	0	0
CH_3—	H—	0.50	10 (50)	50 (30)	60 (20)	2

B. Continuous dye administration

DAB			Percentage of rats with hepatomas after start of dye administration[b]					
R-3'	R-4'	Dose[a]	4 mo.	6 mo.	7 mo.	8 mo.	12 mo.	Activity rank[c]
CH_3—	Cl—	0.50	40 (60)	100				8
Cl—	CH_3—	1.00	0			80 (20)	100	2
Cl—	Cl—	1.00	0			40 (30)	100	1
CH_3—	C_2H_5—	0.25	40 (25)	75	100			10
C_2H_5—	CH_3—	1.00	30 (30)			90	100	4
C_2H_5—	C_2H_5—	0.50	17			33	67	5
C_2H_5—	H—	0.50	10	20		60 (30)	100	6
H—	H—	1.00	25 (30)			75 (15)	100	3
CH_3—	CH_3—	1.00	10 (40)	50	100			7
CH_3—	H—	0.50	90	100				9

[a] Doses are expressed as fractions of "1 dose-equivalent," which was equal to 0.0375 mmoles/day. The daily dose was administered in 0.5 ml corn oil except for 3'-CH_3—, 4'-Cl—, 3'-Cl—, 4'-CH_3—, 3',4'-DiCl—, and 3',4'-DiCH_3-DAB's which, because of lower solubility, were administered in 1 ml.

[b] Percentages are used because groups varied from 10 to 20 animals. Occasionally, a rat died following a laparotomy; it was then not included in the percentages after that time.

[c] Activity or carcinogenic rank was based on the incidence of hepatomas and consideration of the quantity administered (dose equivalent). The larger the rank number, the greater the carcinogenic potency

[d] Percentages of animals with livers that were not normal in appearance at the time of regular laparotomy but which were not scored as carcinogenic.

[e] This animal died 2 days after the 2nd laparotomy with a cirrhotic, nodulated liver, but it was scored negative.

[f] This animal died before 2nd laparotomy; a cyst was found in liver, it was scored negative.

Child et al (1972) reported on the anti-cancer activity of some polyhaloazobenzenes in mice. Mammary adenocarcinomas were transplanted into mice and allowed to grow for 17 days. Then six daily i.p. injections of the test compound were given, followed by excision and weighing of the tumor. Table 125 gives the results, C/T ratio being that of the tumor weight of controls to test mice. Compound (d) was considered marginally active, the others fully active.

TABLE 125.

Pooled results obtained with dose-response tests compounds administered intraperitoneally daily

Compound	Dose in mg./kg.	Number of groups tested	Number of animals	Average C/T activity ratios
(a) 3,3',4,4'-tetrachloroazobenzene	250	3	27	4.62
(b) 3,3'-dichloro-4,4'-difluoroazobenzene	250	2	18	5.92
(c) 3,3',4,4'-tetrabromoazobenzene	250	3	27	4.74
(d) 6-hydroxy 3,3',4,4'-tetrachloroazobenzene	250	4	36	3.40
(e) 2,2',3,3',4,4'-hexachloroazobenzene	250	2	18	10.10

In Table 126 are the results of the same type of experiment, except that an optimal dose and survival time were determined; the figure in the last column includes the 17 day pretreatment, tumor-growth period.

TABLE 126.

Results of survival studies in mice bearing transplanted mammary adenocarcinoma—Administration intraperitoneally once daily

Compound	Optimally effective dose in mg./kg.	Number of groups tested	Number of animals	Median survival times in days at optimally effective dose
3,3',4,4'-tetrachloroazobenzene	81	3	90	89
3,3'-dichloro-4,4'-difluoroazobenzene	9	2	60	38
2,2',3,3',4,4'-hexachloroazobenzene	3	1	30	34

Brown and Kruegel (1972) prepared the six trimethyl DAB derivatives (all three methyls in the "prime" ring) and tested them against DAB for

rat hepatocarcinogenicity. The 3',4',5'-derivative was equivalent to DAB, but the others showed no activity in nine months of testing.

The International Agency for Cancer Research Working Group on the Evaluation of Carcinogenic Risk of Chemicals to Man has been preparing monographs on the following list (finalized in June 1974--private communication) of azo compounds.

Table 127. Azo Compounds Undergoing Evaluation by IARC

DYES

C.I. Acid Orange 10; 1936158*; 1-Naphthalene azo-2',4'-diaminobenzene; C.I. Food Orange 4; Orange G

C.I. Acid Orange 20; 523444*; p-[(4-hydroxy-1-naphthyl)azo]benzene sulphonic acid, sodium salt; Naphthol Orange; Orange I

C.I. Acid Red 2; 493527*; o-[[p-(dimethylamino)phenyl]azo]benzoic acid; Methyl red

C.I. Acid Red 14, disodium salt; 3567699*; C.I. Food Red 3; 2-(4-Sulpho-1-naphthylazo)-1-naphthol-4-sulphonic acid, disodium salt; Carmoisine

C.I. Acid Red 26, disodium salt; 3761533*; 3-Hydroxy-4-(2,4-xylyazo)-2,7-naphthalene disulphonic acid, disodium salt; Ponceau MX; Ponceau 2R

C.I. Acid Red 27; 915673*; 3-Hydroxy-4-[(4-sulpho-1-naphthyl)azo]-2,7-naphthalene disulphonic acid, trisodium salt; Amaranth

C.I. Basic Orange 2; 532821*; 2,4-Diaminoazobenzene-4-hydrochloride; C.I. Solvent Orange 3; Chrysoidine

C.I. Direct Blue 14; 72571*; 3,3'-[(3,3'-Dimethyl-4,4'-biphenylylene)bis(azo)]bis[5-amino-4-hydroxy]-2,7-naphthalenedisulphonic acid, tetrasodium salt; Trypan Blue

C.I. Direct Blue 53; 314136*; 6,6'-[(3,3'-Dimethyl-4,4'-biphenylylene)bis(azo)]bis[4-amino-5-hydroxy-1,3-naphthalenedisulphonic acid tetra sodium salt]; Evans Blue

C.I. Direct Yellow 5; 2852108*; 4'[(6-Hydroxy m tolyl)azo] [illegible]

C.I. Food Red 1, disodium salt; 4548532*; 4-Hydroxy-3-[(5-sulpho-2,4-xylyl)azo]-1-naphthalene sulphonic acid, disodium salt; Ponceau SX; FD + C Red No 4

C.I. Food Red 6; 3564098*; 3-Hydroxy-4-[(2,4,5-trimethylphenyl) azo]-2,7-naphthalenedisulphonic acid, disodium salt; Ponceau 3R

C.I. Food Yellow 3, disodium salt; 2783940*; 6-Hydroxy-5-[(p-sulphophenyl)azo]-2-naphthalenesulphonic acid, disodium salt; Sunset Yellow FCF; FD + C Yellow No 6

C.I. Food Yellow 6, monosodium salt; 2491761*; 4'-Chloro-4-dimethylaminoazobenzene

C.I. Pigment Red 53, barium salt; 5160021*; 5-Chloro-2-[(2-hydroxy-1-naphthyl)azo]-p-toluenesulphonic acid, barium salt; D & C Red No 9

C.I. Solvent Orange 2; 2646175*; 1-(o-Tolylazo)-2-naphthol; Oil Orange SS

C.I. Solvent Orange 7; 3118976*; 1-(2,4-xylylazo)-2-naphthol; Sudan II; Oil Orange XO; and its 2,5-isomer 85825*

C.I. Solvent Red 19; 6368725*; N-Ethyl-1[p-(phenylazo)phenyl] azo]-2-naphthylamine; Sudan Red 7B

C.I. Solvent Red 23; 85869*; 1-[p-(Phenylazo)phenyl]azo]-2-naphthol; Sudan III

C.I. Solvent Red 24; 85836*; 1-[4-(o-Tolylazo)o-tolyl]azo]-2-naphthol; Scarlet Red

C.I. Solvent Red 80; 6358538*; 1-[(2,5-Dimethoxyphenyl)azo]-2-naphthol; Citrus Red No 2

C.I. Solvent Yellow 1; 11021*; p-Aminoazobenzene

C.I. Solvent Yellow 2; 60117*; p-Dimethylaminoazobenzene; Butter Yellow

C.I. Solvent Yellow 3; 97563*; o-Aminoazotoluene

C.I. Solvent Yellow 5; 85847*; 1-(Phenylazo)-2-naphthylamine; Yellow AB

C.I. Solvent Yellow 6; 131703*; 1-(o-Tolylazo)-2-naphthylamine; Yellow OB

C.I. Solvent Yellow 7; 1689823*; 4-Hydroxyazobenzene

C.I. Solvent Yellow 14; 842079*; 1-(Phenylazo)-2-naphthol; Sudan I

Other uses such as pesticides, drugs, etc.

Azobenzene; 103333*; Diphenyldimide

Diacetylaminoazotoluene; 83636*; 4-o-Tolylazo-o-diacetotoluide; Dimazon; Pellidole

p-Dimethylaminobenzenediazo sodium sulphonate; 140567*

Elaiomycin; 499489*; D-threo-methoxy-3-(1-octenyl-ONN-azoxy)-2-butanol

Phenazopyridine; 94780*; 2,6-Diamino-3-phenylazopyridine

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* Chem. Abstract No.

C. Lower Animals

Allen et al (1957) reported that azobenzene was toxic to the cyclamen mite Steneotarsonemus pallidus.

Hayashi et al (1960) in a study of anthelmintics found that Ascaris lumbricoides was susceptible to 1 part in 40,000 of 4-(4-chlorophenylazo)phenol(A), 4-(4-nitrophenylazo)phenol, and 4-(3-nitrophenylazo)phenol(B). Rhabdias bufonis was susceptible, in decreasing order, to A, 4-(4-bromophenylazo)phenol, and B. In vivo in toads a 300-400 mg/kg dose of A was equivalent to an 800 mg/kg dose of 4-iodothymol or of 1-bromo-2-naphthol.

Iwashina (1960) published the following list of azo compounds and their toxicities to earth- and intestinal worms.

Table 128. The Relative Efficacy of Various Organic Compounds against Earthworm and Animal Parasites *in vitro*.

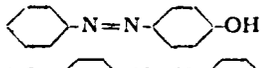
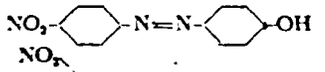
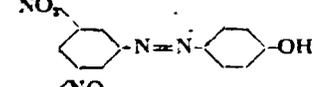
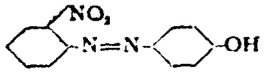
No.	Chemicals (Formula)	Minimum concentration to kill earthworms in 24 hours	Minimum concentration to kill pig ascaris in 24 hours	Minimum concentration to kill toadworms in 24 hours	Minimum concentration to kill larvae of ancylostoma caninum in 24 hours
247		1 : 40,000	1 : 20,000	1 : 40,000	1 : 40,000
255		1 : 80,000	1 : 40,000	1 : 320,000	[1 : 5,000]
264		1 : 160,000	1 : 40,000	1 : 80,000 ~160,000	[1 : 5,000]
265		1 : 80,000	•	[1 : 5,000]	[1 : 5,000]

Table 128. Continued

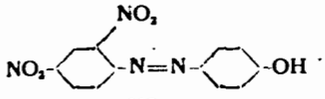
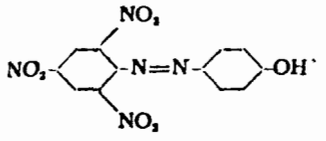
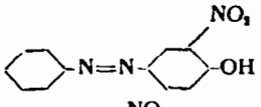
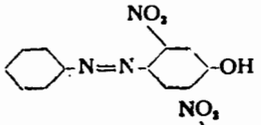
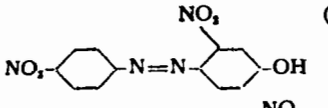
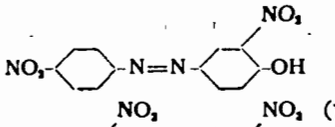
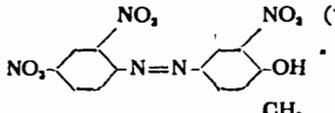
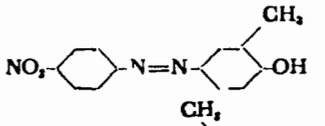
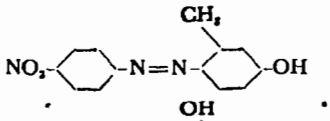
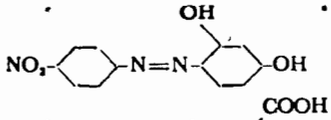
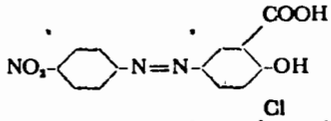
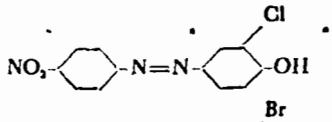
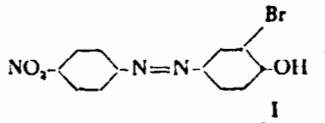
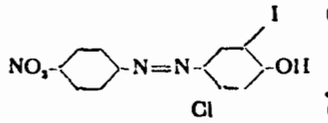
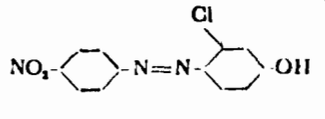
301		1 :	80,000	•	II : 40,000	(1 : 5,000)
302		1 :	160,000	•	II : 20,000	•
303		1 :	160,000	(1 : 5,000)	II : 2,500)	•
304		1 :	160,000	•	II : 20,000	•
305	 (†)	1 :	320,000	•	II : 160,000	(1 : 5,000)
306		1 :	160,000	(1 : 2,500)	II : 2,500	•
307	 (†)	1 :	160,000	•	II : 10,000 —20,000	•
308		1 :	160,000	(1 : 2,500)	II : 5,000)	•
309		1 :	80,000	•	II : 80,000	(1 : 5,000)
310		1 :	160,000	(1 : 2,500)	II : 2,500)	•
311		(1 :	10,000)	•	II : 2,500)	•
312		1 :	160,000	(1 : 2,500)	II : 160,000	(1 : 5,000)
313		1 :	160,000	(1 : 2,500)	II : 160,000	(1 : 5,000)
314	 (†)	1 :	320,000	1 : 2,500	II : 160,000 —320,000	(1 : 5,000)
315	 (†)	1 :	320,000	1 : 2,500	II : 320,000	(1 : 5,000)

Table 128. Continued

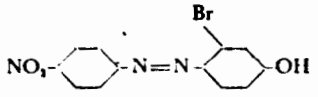
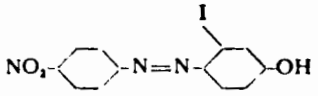
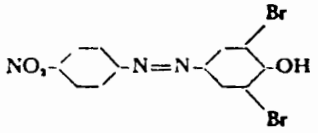
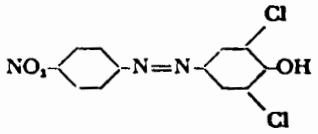
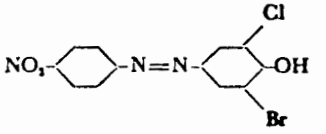
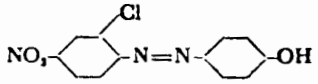
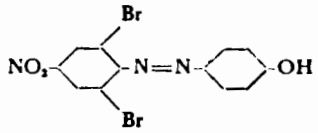
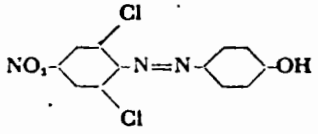
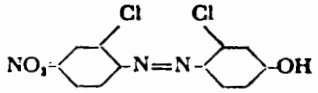
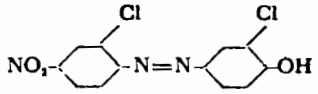
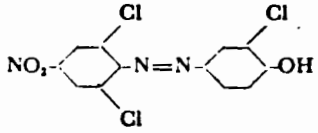
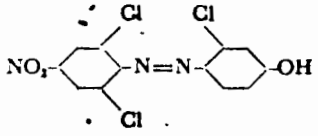
316	 (†)	1 : 80,000	•	1 : 320,000	•
317	 (†)	1 : 5,000	•	1 : 160,000	•
318		1 : 640,000	(1 : 2,500)	(1 : 2,500)	•
319	 (†)	1 : 2,560,000	(1 : 2,500)	1 : 40,000	(1 : 5,000)
320	 (†)	1 : 640,000	(1 : 2,500)	1 : 80,000	(1 : 2,500)
321		1 : 80,000	•	1 : 40,000 ~80,000	(1 : 5,000)
322		1 : 320,000	(1 : 2,500)	1 : 40,000	(1 : 2,500)
323		1 : 80,000	•	•	•
324	 (†)	1 : 320,000	(1 : 2,500)	•	•
325	 (†)	1 : 320,000	•	•	•
326	 (†)	1 : 2,560,000	•	1 : 80,000 ~160,000	(1 : 5,000)
327	 (†)	1 : 1,280,000	•	1 : 160,000	(1 : 5,000)

Table 128. Continued

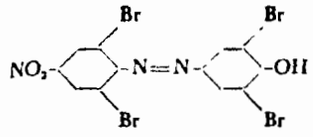
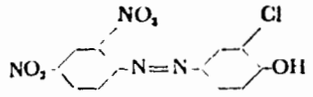
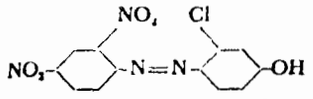
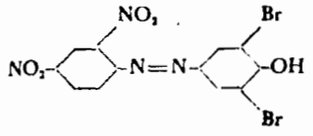
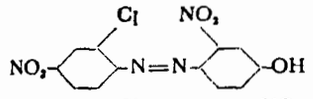
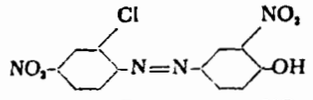
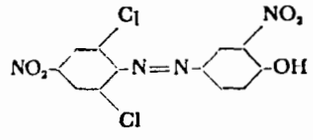
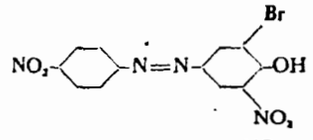
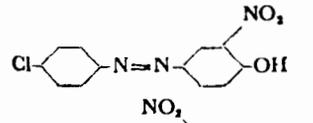
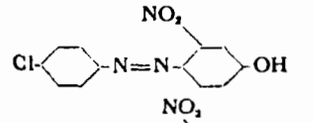
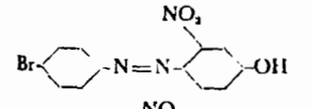
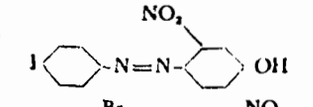
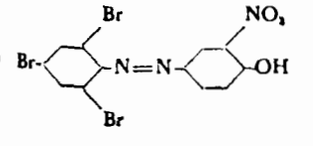
328		(†)	1 : 2,560,000	(1 : 2,500)	(1 : 2,500)	•
329		(†)	1 : 160,000	•	1 : 80,000 ~160,000	(1 : 5,000)
330		(†)	1 : 320,000	•	1 : 80,000	(1 : 5,000)
331			1 : 160,000	•	1 : 80,000	•
332		(†)	1 : 640,000	•	1 : 160,000	(1 : 5,000)
333		(†)	1 : 160,000	•	•	•
334		(†)	1 : 2,560,000	•	1 : 40,000	•
335		(†)	1 : 640,000	(1 : 10,000)	1 : 2,500	•
336		(†)	1 : 40,000	•	(1 : 5,000)	•
337		(†)	1 : 160,000	1 : 2,500)	1 : 40,000	(1 : 2,500)
338		(†)	1 : 320,000	(1 : 2,500)	(1 : 2,500)	•
339		(†)	1 : 40,000	•	•	•
340			1 : 320,000	•	•	•

Table 128. Continued

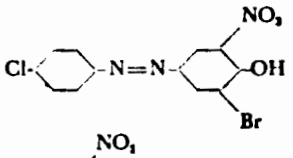
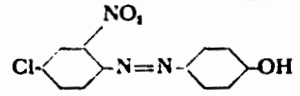
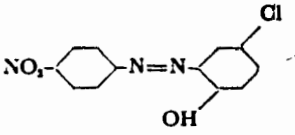
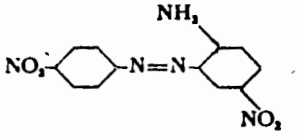
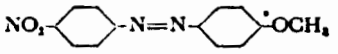
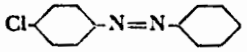
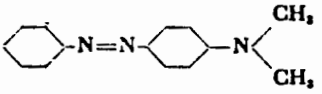
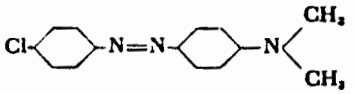
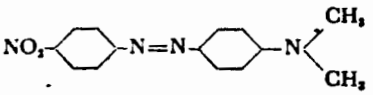
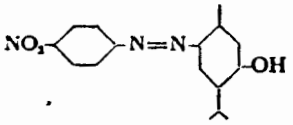
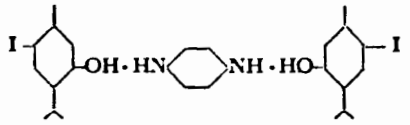
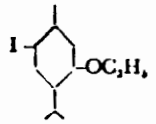
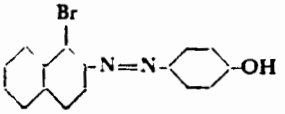
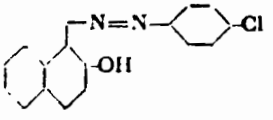
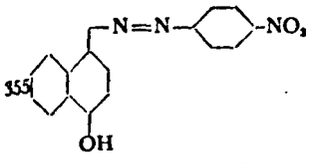
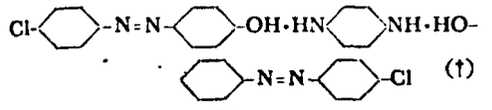
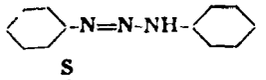
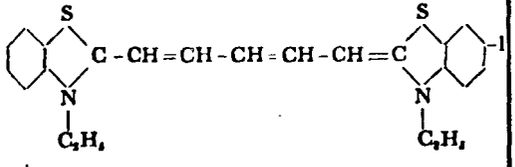
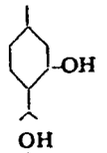
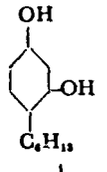
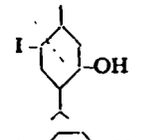
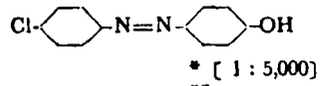
341	 (†)	1 : 1,280,000	•	•	•
342		1 : 80,000	•	(1 : 5,000)	•
343		1 : 2,500	•	•	•
344		(1 : 5,000)	•	(1 : 10,000)	•
345		(1 : 2,500)	•	•	•
346		(1 : 2,500)	•	•	•
347		(1 : 2,500)	•	(1 : 5,000)	•
348		(1 : 2,500)	•	•	•
349		(1 : 2,500)	•	•	•
350		1 : 5,000	•	•	•
351	 (†)	1 : 80,000	1 : 20.000	1 : 80,000	1 : 20,000
352		1 : 5,000	•	•	(1 : 2,500)
353		(1 : 2,500)	•	•	•
354		(1 : 2,500)	•	•	•

Table 128. Continued

355 	[1 : 2,500]	•	[1 : 5,000]	•
356 	1 : 40,000	1 : 20,000	1 : 80,000 ~160,000	1 : 40,000 ~80,000
357 	1 : 20,000	•	1 : 5,000	•
358 	1 : 320,000	[1 : 2,500]	1 : 10,000	[1 : 5,000]
29 	1 : 40,000	1 : 5,000	1 : 20,000	1 : 2,500
183 	1 : 80,000	1 : 40,000	1 : 160,000	1 : 5,000
231 	1 : 80,000 ~160,000	1 : 40,000	1 : 160,000	1 : 20,000
256 	1 : 80,000	1 : 40,000	1 : 320,000	1 : 80,000

* [1 : 5,000] = not effective in the dilution of 1 : 5,000.
** • = not tested.

Jeney and Zsolnai (1964) reported that Ascaris suum, in vitro, was susceptible to these dilutions of various 4-amino-azobenzene (AB) derivatives: 1/10000 for AB, 1/5000 for 2',3-dimethylAB·HCl, 1/5000 for 2,3'-dichloroAB·HCl, 1/5000 for 2-aminoAB·HCl(Chrysoidine), 1/10000 for 4'-chloroAB, and 1/10000 for 4,4'-di-(p-aminophenylazo)biphenyl·2HCl.

Zsolnai (1964) reported that Ascaris suum, in vitro, was susceptible to the dilutions of the various phenylazomalonitrile derivatives in Table 129.

Table 129. Ascaricidal Limiting-Concentrations of Phenyl-azomalonitriles

Phenyl-azo-malonitril	M/ 1,000
2-Tolyl-azo-malonitril	M/ 2,500
3-Tolyl-azo-malonitril	M/ 2,500
4-Tolyl-azo-malonitril	M/ 2,500
2-Chlor-phenyl-azo-malonitril	M/ 1,000
3-Chlor-phenyl-azo-malonitril	M/ 5,000
4-Chlor-phenyl-azo-malonitril	M/ 5,000
4-Brom-phenyl-azo-malonitril	M/ 5,000
4-Jod-phenyl-azo-malonitril	M/ 5,000
4-Aethoxy-phenyl-azo-malonitril	M/ 1,000
2-Methyl-4-brom-phenyl-azo-malonitril	M/ 5,000
2-Methyl-4-jod-phenyl-azo-malonitril	M/10,000
3-Methyl-4-brom-phenyl-azo-malonitril	M/10,000
4-Methyl-2-brom-phenyl-azo-malonitril	M/ 1,000
2-Brom-4-aethoxy-phenyl-azo-malonitril	M/ 1,000
2,5-Dichlor-phenyl-azo-malonitril	M/ 2,500
3,5-Dibrom-phenyl-azo-malonitril	M/10,000
2-Chor-4-brom-phenyl-azo-malonitril	M/10,000
3-Chor-4-brom-phenyl-azo-malonitril	M/10,000
2-Methyl-4,6-dibrom-phenyl-azo-malonitril	M/ 2,500
2-Chlor-4,6-dibrom-phenyl-azo-malonitril	M/10,000
3-Chlor-4,6-dibrom-phenyl-azo-malonitril	M/ 5,000
4-Chlor-2,6-dibrom-phenyl-azo-malonitril	M/ 2,500
2,4,6-Tribrom-phenyl-azo-malonitril	M/ 5,000
2-Nitro-phenyl-azo-malonitril	M/ 1,000
3-Nitro-phenyl-azo-malonitril	M/ 5,000
4-Nitro-phenyl-azo-malonitril	M/10,000
2-Methyl-4-nitro-phenyl-azo-malonitril	M/10,000
3-Methyl-4-nitro-phenyl-azo-malonitril	M/10,000
4-Methyl-2-nitro-phenyl-azo-malonitril	M/10,000
2-Nitro-4-aethoxy-phenyl-azo-malonitril	M/ 1,000
2-Chlor-4-nitro-phenyl-azo-malonitril	M/10,000
3-Chlor-4-nitro-phenyl-azo-malonitril	M/10,000
4-Chlor-2-nitro-phenyl-azo-malonitril	M/10,000
Phenyl-azo-malonitril-4-karbonsäure-aethyl-ester	M/ 2,500
1-Naphtyl-azo-malonitril	M/ 2,500
4-Brom-1-naphtyl-azo-malonitril	M/10,000

Elslager et al (1966) reported on the effectiveness against Schistosoma mansoni in mice of several hundred N-mono- and N,N-dialkyl-N'-(4-arylazo-1-naphthyl)alkylenediamines. Half a dozen of these compounds were tested against the same parasite in rhesus monkeys and found to be effective.

Paulini and Pereira de Souza (1968) tested 3,5-dibromo-4-hydroxy-4'-nitroazobenzene against the eggs, young, and adults of the mollusc Biomphalaria glabrata. LD-50s were 0.15 and 0.11 ppm for 0-1 and 3-4 day eggs, 0.035 ppm for young, and 0.24 ppm for adults; LD-90s were 0.18, 0.17, 0.045, and 0.65 ppm, for the same, respectively.

Balske (1971) patented the use of 4-isothiocyanato-azobenzene as

a treatment for g.i. tract worms in mono- and polygastric animals.

Fahmy and Fahmy (1972) injected male mosquitos, about one day old, with ≤ the LD-30 of various 4-methylaminoazobenzenes and DAB. The males were then allowed to mate with virgin females. Results are in Tables 130, 131, 132, 133, and 134.

TABLE 130.

GENERAL MUTAGENIC ACTIVITIES OF DAB AND ANALOGOUS SUBSTITUTED DERIVATIVES AS INDICATED BY THE YIELD OF X-CHROMOSOME MUTATIONS: RECESSIVE LETHALS AND VISIBLES IN ALL STAGES OF THE TESTIS

Compounds	Dose mM	Chromosomes tested	Mutations		
			lethals	visibles	Total: per 10^3
Controls [1] . . .	—	3707	6(1) [2]	2	2.2±0.8
DAB	10	2070	5(1)	3	3.6±1.1 [3]
	20	986	2(1)	1	
3'-Me-DAB . .	10	1714	2	0	1.2±0.8
3'-ClMe-DAB . .	10	1855	4	0	2.4±0.8
	20	1509	4(1)	0	
N-BzO-MAB . .	10	1706	10(3)	1(1)	6.4±1.9
N-ClEt-MAB . .	10(a)	2036	4(2)	5(1)	4.7±1.2
	10(b)	1337	5	2(1)	
2'-COOH-2-Me-*N*-ClEt-MAB . .	1	1614	13(1)	7	9.4±1.5
	5	2312	17(1)	0	

[1] Males injected with the administration vehicle only: 2% (v/v) dimethylformamide (DMF) in arachis oil.
[2] Number of mosaic (partial tissue) mutants are entered in parentheses.
[3] Mutation frequency in spermatogonial stages was 5.3±2.2 per 10^3.

TABLE 131.

SPECIFIC MUTAGENIC ACTIVITIES OF DAB AND ANALOGOUS SUBSTITUTED DERIVATIVES ON THE *BOBBED (bb)* AND *MINUTE (M)* LOCI

Compounds	Dose mM	Gametes observed	Phenotypic (*bb* + *M*)		Transmitted *bb*		Gametes observed	Transmitted *M*	
			No.	per 10^3	No.	per 10^3		No.	per 10^3
Controls [1] . . .	—	108202	147(5) [2]	1.4±0.11	42(1)	0.4±0.06	207053	17(2)	0.08±0.02
DAB	10	41973	112(6)	2.7±0.5	64(1)	1.5±0.2	81926	5	0.06±0.03
3'-Me-DAB . . .	10	14894	39(5)	2.6±0.4	12(1)	0.8±0.2	28985	1	0.03±0.03
3'-ClMe-DAB . .	10	21749	45(3)	2.1±0.3	2	0.1±0.1	41658	9	0.22±0.04
N-BzO-MAB . .	10	19629	83(1)	4.2±0.5	49(1)	2.5±0.4	36677	4(1)	0.11±0.05
N-ClEt-MAB . .	10	34310	53(3)	1.5±0.2	19	0.6±0.1	34310	1	0.03±0.03
2'-COOH-2-Me-*N*-ClEt-MAB . .	1	5591	10(2)	1.6±0.4	6	0.9±0.3	10737	1(1)	0.13±0.07
	5	6563	10(4)		5		12427	2	

[1] and [2] *See footnote to Table 130.*

Tables 130-134 reprinted with permission from Int. J. Cancer 10:194-206 (1972). Copyright by International Union Against Cancer.

TABLE 132.

MUTAGENIC ACTIVITIES OF DAB AND ANALOGOUS SUBSTITUTED DERIVATIVES WITH RESPECT TO THE *MINUTES* AND X-CHROMOSOME RECESSIVE VISIBLES AS DETECTED BY THE ATTACHED-X TECHNIQUE

Compounds	Dose mM	Gametes tested	X-chromosome visibles	*Minutes*	
				No.	per 10^4
Controls [1]	—	55448	1	37(11)[2]	0.7±0.1
DAB	20	16739	3(1)	9(2)	0.5±0.2
3'-ClMe-DAB .	20	19989	1(1)	9(2)	0.5±0.2
N-BzO-MAB . .	10	14743	4	97(5)	6.6±0.7
N-ClEt-MAB . .	10	17095	0	40(2)	2.3±0.4[3]

[1] and [2] See footnote to Table 130

[3] Mutation frequency in spermatocytic stages was 4.3±1.4 per 10^4.

TABLE 133.

THE EFFECT OF THE CELL STAGE DURING SPERMATOGENESIS ON THE YIELD OF DIFFERENT MUTATIONAL CLASSES WITH DAB AND ANALOGOUS SUBSTITUTED DERIVATIVES

Compounds	Mutational function	Maximal mutagenic response				
		Progeny		Peak mutability relative to mean [1]		
		Sampling days	Main cell stage	Ratio	χ	P
DAB	X-mutations	11-21	Spermatogonia	1.4	0.7	0.2
	bobbed	0-3	Sperm	2.1	3.3	10^{-3}
3'-Me-DAB . . .	*bobbed*	0-3	Sperm	1.4	0.5	0.3
N-BzO-MAB . .	X-mutations	4-6	Spermatids	1.6	0.7	0.2
	bobbed	0-3	Sperm	2.7	4.8	10^{-3}
	Minutes	4-6	Spermatids	1.1	0.7	0.2
N-ClEt-MAB . .	X-mutations	7-10	Spermatocytes	1.5	0.6	0.3
	bobbed	0-3	Sperm	3.8	4.3	10^{-4}
	Minutes	7-10	Spermatocytes	1.9	1.8	0.04
2'-COOH-2-Me-*N*-ClEt-MAB . .	X-mutations	4+5	Spermatids	1.5	1.0	0.2
	bobbed	4+5	Spermatids	2.0	1.2	0.1

[1] Based on χ-combination tests from χ^2 four-fold contingency tables, where the mutation frequencies in the mating periods with the highest yields (derived from the most responsive cells) were compared with the corresponding overall values for the whole progeny representing the mean response of all stages of the testis.

TABLE 134.

SELECTIVITY FOR THE *r*-RNA LOCI WITH DAB AND ANALOGOUS SUBSTITUTED DERIVATIVES AS INDICATED BY THE RELATIVE INDUCTION OF *BOBBED (bb)* AND OTHER X-CHROMOSOME MUTATIONS: RECESSIVE LETHALS AND VISIBLES; X-($l+v$)

Compounds	Induced mutations [1]: per 10^4		Induced *bb* relative to other X-mutations			
	X-($l+v$)	*bb*	Gametes	X-($l+v$) [2]	*bb*	Ratios: *bb*/X-($l+v$)
DAB	1.4±1.3	1.1±0.2	41 973	59	47	0.797±0.052
3'-Me-DAB . . .	0.0	0.4±0.2	14 894	0 (3.7) [3]	6 (13.1) [3]	(3.541)
N-BzO-MAB . .	4.2±2.1	2.1±0.4	19 629	82	41	0.500±0.055
N-ClEt-MAB . .	2.5±1.4	0.2±0.1	34 310	86	5	0.058±0.024
2'-COOH-2-Me-*N*-ClEt-MAB . .	7.2±1.7	0.5±0.3	12 154	88	6	0.068±0.026

[1] The induced frequencies for the various mutational classes were calculated as the weighted means of the observed values from all experiments with the same compound after the subtraction of the corresponding control contributions, based on Tables I and II.

[2] Estimated on the basis of the sample size observed for the *bb*'s and the induced X-mutation frequency.

[3] Upper limits of the Poisson expectations at P = 0.025.

D. Plants

Zsolnai (1964) tested many azo compounds for toxicity to fungi and published the results in the usual form of maximal diluted effective dose (Table 135).

Zsolnai (1965) published some more data on the effectiveness of azo compounds as fungistats (Table 136).

Mitra and Dighe (1968) prepared and tested as fungistats halo- and nitrophenylazosalicyclic acids. In Table 137 Nos. 1-9 refer to R-phenylazo-5-salicyclic acid, where R is 2-nitro, 3-nitro, 4-nitro, 2-chloro, 3-chloro, 4-chloro, 2,4-dichloro, 3-bromo, and 4-bromo.

E. Microorganisms

Koshimura et al (1953) reported the following tubercular-inhibiting, maximum dilutions for substituted azobenzene: 3-methoxy-4-amino-1/40000, 2',3-dimethoxy-4-amino-1/40000, 2',3-dihydroxy-4-cyano-1/40000, 2',3-dimethoxy-4-cyano-1/40000, 2',3-dihydroxy-4-carboxy-1/160000, 2,2'-diacetoxy-1/80000, 2,2',4-trihydroxy-1/64000, 3,5,5'-tribromo-2,2'-dihydroxy-1/20000, 3,5,5'-trichloro-2,2'-dihydroxy-1/160000, 3,5,5'-trichloro-2,2'-diacetoxy-1/160000, 2-hydroxy-5-nitro-4'sulfo-, disodium 1/8000, 4-hydroxy-5-nitro-4'-sulfo-, disodium 1/20000, 4-hydroxy-4'-sulfamyl-1/160000, 4-amino-4'-sulfamyl-1/80000, 4-diethylamino-4'-sulfamyl-1/80000, and 2,2'-dihydroxy-1/320000.

Raynaud et al (1957) tested some azobenzenes both in vitro and in vivo against Myobacterium tuberculosis. The following were the only ones to show in vivo activity: 2-methyl-4-hydroxy-5-isopropyl-4'-sulfonamido-, 2-methyl-3', 4-dihydroxy-5-isopropyl-4'-carboxy-, and 2-amino-4-hydroxy-5-carboxy-4'-sulfonamido-. Finkelstein (1961) tested

Table 135. Azo Compounds as Fungistats

Fungus	Phenyl-azo-malo-nitril F 2201	2-Tolyl-azo-malo-nitril F 2202	3-Tolyl-azo-malo-nitril F/2203	4-Tolyl-azo-malo-nitril F/2204	2-Chlor-phenyl-azo-malo-nitril F/2205	3-Chlor-phenyl-azo-malo-nitril F/2206	4-Chlor-phenyl-azo-malo-nitril F/2207	4-Brom-phenyl-azo-malo-nitril F/2208	4-Jod-phenyl-azo-malo-nitril F/2209	4-Aethoxy-phenyl-azo-malo-nitril F 2210	2-Methyl-4-brom-phenyl-azo-malo-nitril F/2211
Candida albicans	M 2.5[illegible]	M 5[illegible]	M/ 2.500	—	M 5,000	M/10.000	M/10.000	M 10,000	M/ 1,000	—	M 5.000
Cryptococcus ruber	M 5.000	M 5[illegible]	M 5,000	M/ 5,000	M/ 5.000	M/25.000	M/50,000	M 50,000	M/25.000	—	M 5.000
Saccharomyces cerevisiae	M 2.500	M 5.000	M/ 2,500	—	M/ 2,500	M/ 5,000	M/10,000	M 10.000	M/ 1.000	—	M 5.000
Trichophyton gypseum	M 25.000	M 25.000	M/25.000	M/25,000	M/25,000	M/25.000	M/25.000	M/25.000	M/50.000	M/25,000	M 25.000
Epidermophyton Kaufman-Wolff	M 25.000	M 50.000	M 50.000	M 50.000	M 50.000	M/50.000	M/50.000	M 50.000	M 50.000	M 50.000	M 25.000
Achorion quinckeanum	M 25.000	M 50.000	M 50.000	M 25.000	M 25.000	M/50.000	M/50.000	M 50.000	M/50.000	M 50.000	M 25.000
Trichothecium roseum	M 10.000	M 25.000	M 25.000	M/10.000	M 10.000	M/25.000	M/25.000	M 25.000	M 25.000	M 25.000	M 25.000
Penicillium janthinellum	M 2.500	M 5.000	M 5.000	—	M 2.500	M/ 5.000	M/10.000	M 10.000	—	—	M 10,000
Penicillium simplicissimum	M 5.000	M 5.000	M/ 5,000	M/ 1,000	M 2,500	M/10.000	M/10.000	M/25.000	M/25.000	—	M 5.000
Aspergillus niveus	M 2.500	M 2.500	M 2,500	—	M 2,500	M/10.000	M/10.000	M 25.000	M 25.000	—	M 2.500
Aspergillus elegans	M 2.500	M 1.000	M/ 2.500	—	—	M/ 5,000	M/ 5,000	M/ 1,000	—	—	M/ 1.000
Aspergillus niger	M 2.500	M 2.500	M 2,500	M/ 2,500	M/ 2.500	M/10.000	M/25.000	M/25.000	M/ 5.000	M 5.000	M 5.000
Actinomucor repens	M 2.500	M 1.000	M 2.500	—	M 2.500	M/ 5.000	M/10.000	—	—	—	M 5.000
Botrytis cinerea	M 2.500	M 2.500	M 2.500	—	M/ 2,500	M/ 5,000	M/10.000	—	—	—	—
Fusarium oxysporum	M 2.500	M 2.500	M 2.500	—	M/ 2.500	M/ 5.000	M/10.000	—	—	—	M 2.500
Fusarium solani	M 2.500	M 2.500	M 2.500	—	M/ 2.500	M/ 5.000	M/ 5.000	—	—	—	M/ 2.500

Table 135. Continued

	2-Methyl-4-jod-phenyl-azo-malo-nitril F 2212	3-Methyl-4-brom-phenyl-azo-malo-nitril F 2213	4-Methyl-2-brom-phenyl-azo-malo-nitril F/2214	2-Brom-4-aethoxy-phenyl-azo-malo-nitril F/2215	2,5-Di-chlor-phenyl-azo-malo-nitril F/2216	3,5-Di-brom-phenyl-azo-malo-nitril F/2217	2-Chlor-4-brom-phenyl-azo-malo-nitril F/2218	3-Chlor-4-brom phenyl-azo-malo-nitril F/2219	2-Methyl-4,6-di-brom-phenyl-azo-malo-nitril F 2220	2-Chlor-4,6-di-brom-phenyl-azo-malo-nitril F/2221	3-Chlor-4,6-di-brom-phenyl-azo-malo-nitril F 2222
	M 50.000	M/50.000	M/25,000	M/25,000	M/50,000	M/50,000	M/50,000	M/50,000	M/50,000	M/50,000	M 50 000
	M 50.000	M 50.000	M/25,000	M/25,000	M/50,000	M/50,000	M/50,000	M 50.000	M/50.000	M/50,000	M 50,000
	M 50.000	M 50.000	M/25.000	M/25,000	M/50,000	M/50,000	M/50,000	M/50.000	M/50.000	M 50,000	M 50,000
	M 5.000	M/ 5.000	M/ 2.500	M/ 1 000	M/ 5.000	M/25.000	M/10,000	M/10.000	M/ 2.500	M/ 2,500	M/ 5 000
	M 5.000	M 5.000	M/ 2.500	M/ 1,000	M/ 5.000	M/10.000	M/ 5.000	M/ 5.000	M/ 2.500	M/ 1.000	M 2.500
	M 10.000	M 10.000	M/10.000	M/ 5.000	M 10.000	M/25.000	M/25.000	M 25.000	M 10.000	M 10.000	M 10.000
	M 5.000	M 10.000	M/ 5 000	M/ 2,500	M/ 5.000	M 10.000	M 10,000	M 10.000	M 5.000	M 2,500	M 2,500
	M 5 000	M 5.000	M/ 2.500	M/ 1.000	M/ 5.000	M/ 5,000	M/ 5.000	M 5.000	M 2 500	M 1.000	M 2.500
	M 5.000	M 5.000	M/ 2,500	M/ 1.000	M 5.000	M/ 5.000	M/ 5.000	M 5.000	M 2 500	M 1.000	M 1.000
	M 5.000	M 5.000	M 2,500	M/ 1,000	M 5.000	M/ 5.000	M/10.000	M 5 000	M 2,500	M 1.000	M 2,500
	M 2,500	M 2,500	M/ 1.000	—	M 2,500	M 2,500	M/ 2 500	M 2.500	M 2.500	M 1.000	M 1.000
	M 2,500	M/ 2.500	M/ 1,000	—	M/ 2,500	M/ 2,500	M/ 2.500	M/ 2.500	M 2 500	M 1,000	M 1.000
Candida albicans	M 2,500	M 2,500	M/ 5,000	—	M 5.000	—	M/10.000	M 5.000	500	M/ 2,500	M 5.000
Cryptococcus ruber	M 5.000	M/ 5.000	M/ 5.000	—	M 10,000	M 10.000	M/10.000	M 10.000	M/ 000	M 10.000	M 5.000
Saccharomyces cerevisiae	M 2,500	M 1.000	M/ 5.000	—	M/ 5.000	M/ 1,000	M/10,000	M 10.000	M 2 500	M 2,500	M 5.000
Trichophyton gypseum	M 10,000	M 50.000	M/25,000	M/50,000	M 50,000	M 50,000	M/50.000	M 50.000	M 25,000	M 25.000	M 50.000
Epidermophyton Kaufman-Wolff	M 10 000	M 50.000	M 25,000	M/50,000	M 50.000	M 50,000	M 50,000	M 50.000	M 25.000	M 25.000	M 50 000
Achorion quinckeanum	M 25.000	M 50.000	M 25.000	M/50.000	M 50.000	M 50.000	M 50.000	M 50 000	M 25 000	M 25.000	M 50 000
Trichothecium roscum	M 25.000	M 25.000	M 25.000	M/50,000	M 50.000	M 50.000	M 25,000	M 25.000	M 25 000	M 25.000	M 25.000
Penicillium javanicum	M 5.000	M 5.000	M 5.000	—	M 5.000	M 10.000	M 10.000	M 10.000	M 5.000	M 5.000	M 10.000
Penicillium simplicissimum	M 2.500	M 5.000	M/ 2.500	—	M 10.000	M/10.000	M/10.000	M 10 000	M 5 000	M 5 000	M 10.000
Aspergillus niveus	M 2,500	M 5,000	M 2,500	—	M 2.500	M/10.000	M 10,000	M 10.000	M 5.000	M 2,500	M 5.000
Aspergillus elegans	—	—	M/ 1.000	—	M 1,000	M/ 1,000	M/ 2,500	M 2.500	M 1.000	M 1.000	M 1.000
Aspergillus niger	M 2.500	M 5.000	M/ 2,500	—	M 10.000	M 5,000	M/ 5,000	M 10.000	M 5.000	M 5.000	M 5.000
Actinomucor repens	—	—	M/ 1.000	(M/ 1,000)	M 2.500	—	M/ 2.500	—	M 1.000	M 5.000	—
Botrytis cinerea	—	—	—	(M/ 1,000)	M 1.000	—	M 1.000	—	M 1.000	M 2.500	—
Fusarium oxysporum	—	—	—	(M/ 1,000)	M 1.000	—	M/ 1,000	—	M 1.000	M 2.500	—
Fusarium solani	—	—	(M/ 1.000)	—	M 2.500	—	M 1.000	—	M 1.000	M 5.000	—

Table 135. Continued

	4-Chlor-2,6-di-brom-phenyl-azo-malo-nitril F/2223	2,4,6-Tribrom-phenyl-azo-malo-nitril F/2224	2-Nitro-phenyl-azo-malo-nitril F/2225	3-Nitro-phenyl-azo-malo-nitril F/2226	4-Nitro-phenyl-azo-malo-nitril F/2227	2-Methyl-4-nitro-phenyl-azo-malo-nitril F/2228	3-Methyl-4-nitro-phenyl-azo-malo-nitril F/2229	4-Methyl-2-nitro-phenyl-azo-malo-nitril F 2230	2-Nitro-4-aethoxy-phenyl-azo-malo-nitril F 2231	2-Chlor-4-nitro-phenyl-azo-malo-nitril F 2232	3-Chlor-4-nitro-phenyl-azo-malo-nitril F 2233
	M 50,000	M 50.000	M/10,000	M/10,000	M/10,000	M/10.000	M/10,000	M/10.000	M/10.000	M 10.000	M 50.000
	M 50.000	M 50.000	M/10,000	M/10,000	M/10.000	M/10,000	M/10,000	M 10.000	M/10,000	M/10.000	M 50,000
	M 50,000	M 50.000	M/10,000	M/10,000	M/10.000	M/10,000	M/10,000	M/10.000	M/10,000	M 10,000	M 25.000
	M 2,500	M 2.500	M/ 5,000	M/ 5,000	M/ 5.000	—	M/ 1.000	M/ 2.500	M/ 1.000	M/ 2,500	M/ 5.000
	M 2.500	M/ 2.500	M/ 5,000	M/ 2,500	M/ 2.500	—	M/ 1,000	M 2.500	M/ 1.000	—	M 2.500
	M 5.000	M 5.000	M 25,000	M/10,000	M 10.000	M/ 5.000	M/ 5.000	M 5.000	M 2,500	M/ 2,500	M 10.000
	M 2.500	M 2.500	M/10,000	M/ 2,500	M/ 2.500	M/ 1,000	M/ 1,000	M 2.500	M 1.000	M/ 1,000	M 2.500
	M 1,000	M/ 1.000	M/ 5.000	M/ 2,500	M/ 1,000	—	—	M/ 2.500	M/ 1.000	—	M 2.500
	M 1,000	M/ 1.000	M/ 5,000	M/ 2,500	M/ 1,000	—	—	M 2,500	M/ 1,000	—	M 2.500
	M 2.500	M/ 1.000	M/ 5,000	M/ 2,500	M/ 1,000	—	—	M 1,000	M/ 1.000	—	M 2.500
	M/ 1.000	M 1.000	—	—	—	—	—	—	—	—	M 1.000
	M/ 1,000	M/ 1.000	—	—	—	—	—	—	—	—	M 1,000
Candida albicans	M/ 2.500	M 2.500	M/ 1,000	—	—	M/ 2,500	M/ 1,000	M 2.500	—	M/ 2,500	M 1.000
Cryptococcus ruber	M 5,000	M 10.000	M/ 5,000	M/ 1,000	M/ 1,000	M/ 2,500	M/ 1,000	M 5.000	—	M 2,500	M 2.500
Saccharomyces cerevisiae	M 2,500	M 2.500	M/ 1,000	—	—	M/ 2,500	M/ 1.000	M 2.500	—	M/ 2,500	M 1.000
Trichophyton gypseum	M 25,000	M 25.000	M/25,000	M/10,000	M/ 5.000	M 25,000	M/25,000	M 50.000	M 25,000	M 50,000	M 50.000
Epidermophyton Kaufman-Wolff	M 25.000	M 25.000	M/25,000	M/10,000	M/10.000	M/25,000	M 50,000	M 50 000	M 50.000	M 50 000	M 50.000
Achorion quinckeanum	M 25i000	M 25 000	M/25.000	M/10,000	M/ 5,000	M 25.000	M/50.000	M 50 000	M 50.000	M 50,000	M 50.000
Trichothecium roseum	M 25,000	M 25 000	M/25,000	M/10,000	M/10,000	M 10.000	M/10,000	M 25 000	M 25.000	M 25,000	M 25.000
Penicillium javanicum	M 2,500	M 5.000	M/ 5.000	——	—	M/ 1,000	M/ 1,000	M/ 2.500	M/ 1,000	M 1,000	M/ 1.000
Penicillium simplicissimum	M 2,500	M/ 2.500	M/ 5,000	—	M/ 1.000	M/ 2,500	M/ 1,000	M 2,500	M/ 1.000	M 2.500	M 2.500
Aspergillus niveus	M 2.500	M 2.500	—	—	M/ 1.000	M/ 1,000	M/ 1,000	—	—	M 2,500	—
Aspergillus elegans	M 1,000	M 1.000	—	—	M/ 1,000	M 1.000	M/ 1.000	—	—	M 2.500	—
Aspergillus niger	M 5.000	M 5.000	—	M/ 1,000	M/ 1,000	M/ 1,000	M/ 1,000	M/ 2.500	—	M/ 2,500	M 1.000
Actinomucor repens	M 2.500	M 1.000	M/ 1,000	—	—	—	—	—	—	(M 1000)	—
Botrytis cinerea	M 1.000	M 1.000	M 2.500	—	M/ 1,000	M/ 1.000	—	—	—	M 1.000	M/ 1.000
Fusarium oxysporum	M 1.000	M 1.000	M/ 2,500	—	—	M 2.500	(M,1000)	M 1.000	—	M 1,000	M 1.000
Fusarium solani	M 1.000	M 1.000	M/ 2,500	—	M/ 1.000	M/ 2.500	(M,1000)	M 1.000	—	M/ 1,000	M 1.000

Table 135. Continued

	Phenyl-azo-acet-essig-ester F/2271	3-Tolyl-azo-acet-essig-ester F/2272	4-Tolyl-azo-acet-essig-ester F/2273	3-Chlor-phenyl-azo-acet-essig-ester F/2274	4-Chlor-phenyl-azo-acet-essig-ester F/2275	Phenyl-azo-malon-saure diaethyl-ester F/2276	4-Tolyl-azo-malon-saure-diaethyl-ester F/2277	4-Chlor-phenyl-azo-malonsaure diaethyl-ester F 2278
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
Candida albicans	—	—	—	—	—	—	—	—
Cryptococcus ruber	—	—	—	—	—	(M 1000)	—	M 1.000
Sacharomyces cerevisiae	—	—	—	—	—	—	—	—
Trichophyton gypseum	M 10.000	M/10,000	M/10.000	M 10.000	M 10,000	M 2.500	M/ 2,500	M 5,000
Epidermophyton Kaufman-Wolff	M/10.000	M/10,000	M/10.000	M/10,000	M 10,000	M 2.500	M/ 2,500	M 5,000
Achorion quinckeanum	M/10.000	M/10.000	M 10.000	M/10.000	M 10.000	M/ 2.500	M/ 2.500	M 5.000
Trichothecium roseum	M/ 5.000	M/ 5,000	M 10.000	M 10.000	M/10,000	M 1.000	M/ 1.000	M 2.500
Penicillium javanicum	—	—	(M/1000)	(M/1000)	M/ 1,000	—	—	—
Penicillium simplicissimum	—	—	M/ 1.000	M/ 1.000	M/ 1,000	—	—	—
Aspergillus niveus	—	—	—	—	—	—	—	—
Aspergillus elegans	—	—	—	—	—	—	—	—
Aspergillus niger	—	—	(M 1000)	(M/1000)	(M/1000)	—	—	—
Actinomucor repens	M/ 1.000	—	—	—	M/ 1,000	—	—	—
Botrytis cinerea	(M 1000)	—	—	—	M 1,000	—	—	—
Fusarium oxysporum	(M 1000)	—	—	—	M/ 1,000	—	—	—
Fusarium solani	M 1.000	—	—	—	M/ 1.000	—	—	—

A (–) means that a concentration of M/1000 had no effect
(M/1000), but not M/1000, means that this concentration had a partial effect

Table 135. Continued

	4-Chlor-phenyl-azo-cyan-essig-säure-aethyl-ester F 2253	Phenyl-azo-cyan-essig-saure-anilid F/2257	4-Chlor-phenyl-azo-cyan-essig-säure-anilid F/2259	Phenyl-azo-cyan-essig-säure-(4'-chlor-anilid) F/2260	4-Chlor-phenyl-azo-cyan-essig-säure-(4'-chlor-anilid) F/2262	Phenyl-azo-cyan-essig-säure-hydrazid F/2263	4-Tolyl-azo-cyan-essig-saure-hydrazid F/2264	4-Chlor-phenyl-azo-cyan-essig-säure-hydrazid F/2265	Phenyl-azo-acetyl-aceton F 2266	3-Tolyl-azo-acetyl-aceton F 2267
	—	—	—	—	—	—	—	M/ 5,000	—	—
	—	—	—	—	—	—	—	M/ 5,000	—	—
	—	—	—	—	—	—	—	M/ 5,000	—	—
	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—
Candida albicans	—	—	—	—	—	—	—	—	—	—
Cryptococcus ruber	—	—	—	—	—	—	—	M/ 1,000	—	—
Saccharomyces cerevisiae	—	—	—	—	—	—	—	—	—	—
Trichophyton gypseum	M 25.000	M/ 1,000	M/ 1,000	M/ 1,000	M/ 1,000	(M/1000)	M/ 1,000	M/ 5,000	M 2.500	M 5.000
Epidermophyton Kaufman-Wolff	M 25.000	M/ 2,500	M/ 1,000	M/ 2,500	M/ 1,000	M/ 1,000	M/ 1,000	M/ 5,000	M 2.500	M 5.000
Achorion quinckeanum	M 25.000	M/ 2,500	M/ 1,000	M/ 2,500	M/ 1,000	(M/1000)	M/ 1,000	M/ 5,000	M 2.500	M 5.000
Trichothecium roseum	M 5,000	(M/1000)	—	(M/1000)	—	—	M/ 1,000	M/ 2,500	M 2.500	M 5.000
Penicillium javanicum	—	—	—	—	—	—	—	(M/1000)	—	—
Penicillium simplicissimum	—	—	—	—	—	—	—	(M/1000)	—	—
Aspergillus niveus	—	—	—	—	—	—	—	—	—	—
Aspergillus elegans	—	—	—	—	—	—	—	—	—	—
Aspergillus niger	—	—	—	—	—	—	—	—	—	—
Actinomucor repens	—	—	—	—	—	—	—	—	—	—
Botrytis cinerea	—	—	—	—	—	—	—	—	—	—
Fusarium oxysporum	—	—	—	—	—	—	—	—	—	—
Fusarium solani	—	—	—	—	—	—	—	—	—	—

Table 135. Continued

	4-Chlor-2-nitro-phenyl-azo-malo-nitril F 2234	Phenyl-azo-malo-nitril-4-karbon-saure-aethyl-ester F 2238	1-Naphtyl-azo-malo-nitril F 2243	4-Brom-1-naph-tyl-azo-malo-nitril F/2244	Azoben-zol-4-azo-malo-nitril F/2245	3,3'-Di-methyl-diphenyl-en-4,4'-bis-(azo-malo-nitril F/2247	Phenyl-azo-cyan-essig-säure methyl-ester F/2248	4-Tolyl-azo-cyan-essig-säure methyl-ester F/2249	4-Chlor-phenyl-azo-cyan-essig-säure-methyl-ester F/2250	Phenyl-azo-cyan-essig-säure-aethyl ester F 2251	4-Tolyl-azo-cyan-essig-säure-aethyl-ester F 2252
	M 50.000	M 25.000	M 50.000	M/50,000	M/50,000	M 10,000	—	—	—	—	—
	M 50.000	M 25.000	M 50.000	M/50,000	M/50.000	M/10,000	—	—	—	—	—
	M 50.000	M 25.000	M 50.000	M/50,000	M/50,000	M/10,000	—	—	—	—	—
	M 5.000	M 1.000	M 5.000	M/ 5,000	—	—	—	—	—	—	—
	M 2.500	M/ 1.000	M/ 2,500	M/ 2,500	—	—	—	—	—	—	—
	M 25.000	M 5.000	M 10.000	M/10,000	—	—	—	—	—	—	—
	M 5.000	M 2.500	M 5.000	M 5,000	—	—	—	—	—	—	—
	M 5.000	M 2.500	M 2.500	M/ 2,500	—	—	—	—	—	—	—
	M 2.500	M 2.500	M 2.500	M/ 2,500	—	—	—	—	—	—	—
	M 2.500	M 2.500	M 2.500	M/ 5.000	—	—	—	—	—	—	—
	—	—	M 1.000	M/ 1.000	—	—	—	—	—	—	—
	—	—	M 1.000	M/ 1.000	—	—	—	—	—	—	—
Candida albicans	M 2.500	—	M 1.000	M/ 1,000	—	—	—	—	—	—	—
Cryptococcus ruber	M 5.000	M 5.000	M/ 5,000	M/ 2,500	—	—	—	—	—	—	—
Saccharomyces cerevisiae	M 2.500	—	M 1.000	M/ 1,000	—	—	—	—	—	—	—
Trichophyton gypseum	M 50.000	M 25.000	M 10.000	M/10.000	M/ 2,500	—	M/ 5,000	M/10,000	M/25,000	M 10.000	M 10.000
Epidermophyton Kaufman-Wolff	M 50.000	M 25.000	M/25.000	M/25.000	M/ 5,000	—	M/10,000	M/10,000	M/25,000	M 10.000	M 10.000
Achorion quinckeanum	M 50.000	M 25.000	M 25.000	M/25,000	M/ 5,000	—	M/10,000	M/10.000	M/25,000	M 10.000	M 10.000
Trichothecium roseum	M 25.000	M 25.000	M 25.000	M/25,000	M/ 5,000	—	M/ 2,500	M/ 5,000	M/10,000	M 5.000	M 5.000
Penicillium javanicum	M 2.500	—	M/ 5.000	M/ 5,000	—	—	—	—	—	—	—
Penicillium simplicissimum	M 5.000	—	M 5.000	M/ 5,000	—	—	—	—	—	M 2.500	—
Aspergillus niveus	M 1.000	—	M 1.000	M/ 2,500	—	—	—	—	—	M 1.000	—
Aspergillus elegans	M 1.000	—	M 1.000	M/ 1,000	—	—	—	—	—	M 1.000	—
Aspergillus niger	M 2.500	—	M/ 1.000	M/ 2,500	—	—	—	—	—	—	—
Actinomucor repens	M 1.000	—	—	—	—	—	—	—	—	—	—
Botrytis cinerea	M 2.500	—	—	—	—	—	—	—	—	—	—
Fusarium oxysporum	M 2.500	—	—	—	—	—	—	—	—	—	—
Fusarium solani	M 2.500	—	—	—	—	—	—	—	—	-	-

Table 136. Azo Compounds as Fungistats

Fungus	4-Acetyl-phenyl-azo-malonitril F/2313	4-Acetyl-phenyl-azo-malonitril-semicarbazon F/2318	4-Acetyl-phenyl-azo-malonitril-oxym F/2321	4-Acetyl-phenyl-azo-malonitril-phenyl-hydrazon F/2322	4-Acetyl-phenyl-azo-malonitril-salizyloyl-hydrazon F/2323	4-Acetyl-phenyl-azo-malonitril-isonikotinoyl-hydrazon F/2324	4-Acetyl-phenyl-azo-cyanessigsäure-methyl-ester-amino-guanidon hydrochlorid F/2328	4-(4'-Acetyl-phenyl-azo)-3,5-dimethyl-pyrazolyl-1-amidin-aminoguanidon dihydrochlorid F/2330
	M/10,000	M/ 2,500	M/ 1 000	M/10,000	M/ 5,000	M/ 2,500	M/ 2,500	M/10,000
	M/10,000	M/ 2,500	M/ 1,000	M/10,000	M/ 5,000	M/ 2,500	M/ 2,500	M/10,000
	M/ 5,000	M/ 2,500	(M/1000)	M/ 5,000	M/ 5,000	(M/1000)	M/ 1,000	M/10,000
	M/ 5,000	M/ 2,500	M/ 1,000	M/ 5,000	M/ 2,500	M/ 1,000	M/ 1,000	—
	M/ 5,000	M/ 1,000	—	M/ 2,500	M/ 2,500	—	—	—
	M/10,000	M/ 2,500	—	M/ 5,000	M/ 2,500	—	—	—
	M/ 5,000	M/ 1,000	—	M/ 1,000	—	—	—	—
	M/ 1,000	M/ 1,000	—	M/ 1,000	—	—	—	—
	M/ 1,000	—	—	M/ 1,000	—	—	—	—
	M/ 1,000	—	—	M/ 1,000	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
Fungus								
Candida albicans	—	—	—	—	—	—	—	—
Cryptococcus ruber	M/ 2,500	M/ 2,500	—	M/ 2,500	—	M/ 2,500	—	—
Saccharomyces cerevisiae	—	—	—	—	—	—	—	—
Trichophyton gypseum	M/ 2,500	M/ 2,500	—	M/ 5,000	—	M/ 2,500	M/ 1,000	—
Epidermophyton Kaufman-Wolff	M/ 2,500	M/ 2,500	—	M/ 5,000	—	M/ 2,500	M/ 2,500	—
Achorion quinckeanum	M/ 2,500	M/ 2,500	—	M/ 5,000	—	M/ 2,500	M/ 2,500	—
Trichothecium roseum	M/ 2,500	M/ 2,500	—	M/ 2,500	—	M/ 2,500	M/ 2,500	—
Penicillium javanicum	—	—	—	—	—	—	—	—
Penicillium simplicissimum	—	—	—	—	—	—	—	—
Aspergillus niveus	—	—	—	—	—	—	—	—
Aspergillus elegans	—	—	—	—	—	—	—	—
Aspergillus niger	—	—	—	—	—	—	—	—
Actinomucor repens	—	—	—	—	—	—	—	—
Botrytis cinerea	—	—	—	—	—	—	—	—
Fusarium oxysporum	—	—	—	—	—	—	—	—
Fusarium solani	—	—	—	—	—	—	—	—

Table 136. Continued

	4-(4'-Acetyl-phenyl-azo)-3-methyl-pyrazolon(5)-yl-1-amidin-amino-guanidon dihydrochlorid F/2331	4-Phenyl-azo-3,5-dimethyl-pyrazolyl-1-amidin hydrochlorid F/2344	4-(3'-Tolyl-azo)-3,5-dimethyl-pyrazolyl-1-amidin hydrochlorid F/2345	4-(4'-Tolyl-azo)-3,5-dimethyl-pyrazolyl-1-amidin hydrochlorid F/2346	4-(3'-Chlor-phenyl-azo)-3,5-dimethyl-pyrazolyl-1-amidin hydrochlorid F/2347	4-(4'-Chlor-phenyl-azo)-3,5-dimethyl-pyrazolyl-1-amidin hydrochlorid F/2348	4-(4'-Tolyl-azo)-3,5-dimethyl-pyrazolyl-1-thiocarbon-säureamid F/2356	4-(3'-Chlor-phenyl-azo)-3,5-dimethyl-pyrazolyl-1-thiocarbon-säureamid F/2357
	M/ 5,000	M/ 5,000	M/ 5,000	M/10,000	M/10,000	M/10,000	M/ 2,500	M/ 2,500
	M/ 5,000	M/ 2,500	M/ 5,000	M/10,000	M/10 000	M/10,000	M/ 2,500	M/ 2,500
	M/ 2,500	M/ 5,000	M/ 5,000	M/10,000	M/10,000	M/10,000	M/ 2,500	M/ 2,500
	—	M/ 2,500	M/ 2,500	M/ 2,500	M/ 2,500	M/ 2,500	—	—
	—	M/ 1,000	M/ 1,000	M/ 1,000	(M/1000)	M/ 1,000	—	—
	—	M/ 1,000	M/ 1,000	M/ 1,000	(M/1000)	M/ 1,000	—	—
	—	M/ 1,000	M/ 1,000	M/ 1,000	(M/1000)	M/ 1,000	—	—
	—	M/ 1,000	M/ 1,000	M/ 1,000	—	M/ 1,000	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
Candida albicans	—	—	—	—	—	—	—	—
Cryptococcus ruber	—	(M/1000)	(M/1000)	(M/1000)	(M/1000)	(M/1000)	—	—
Saccharomyces cerevisiae	—	—	—	—	—	—	—	—
Trichophyton gypseum	—	M/ 5,000	M/ 5,000	M/ 5,000	M/10,000	M/10,000	M/ 5,000	M/ 5,000
Epidermophyton Kaufman-Wolff	—	M/ 5,000	M/ 5,000	M/ 5,000	M/10,000	M/10,000	M/ 5,000	M/ 5,000
Achorion quinckeanum	—	M/ 5,000	M/ 5,000	M/ 5,000	M/10,000	M/10,000	M/ 5,000	M/ 5,000
Trichothecium roseum	—	M/ 1,000	M/ 1,000	M/ 1,000	M/ 2,500	M/ 2,500	M/ 5,000	M/ 5,000
Penicillium javanicum	—	—	—	—	—	—	—	—
Penicillium simplicissimum	—	—	—	—	—	—	—	—
Aspergillus niveus	—	—	—	—	—	—	—	—
Aspergillus elegans	—	—	—	—	—	—	—	—
Aspergillus niger	—	—	—	—	—	—	—	—
Actinomucor repens	—	—	—	—	—	—	—	—
Botrytis cinerea	—	—	—	—	—	—	—	—
Fusarium oxysporum	—	—	—	—	—	—	—	—
Fusarium solani	—	—	—	—	—	—	—	—

Table 136. Continued

	4-(4'-Chlorphenyl-azo)-3,5-dimethylpyrazolylthiocarbonsäureamid F/2358
Candida albicans	—
Cryptococcus ruber	—
Saccharomyces cerevisiae	—
Trichophyton gypseum	M/ 5,000
Epidermophyton Kaufman-Wolff	M/ 5,000
Achorion quinckeanum	M/ 5,000
Trichothecium roseum	M/ 5,000
Penicillium javanicum	—
Penicillium simplicissimum	—
Aspergillus niveus	—
Aspergillus elegans	—
Aspergillus niger	—
Actinomucor repens	—
Botrytis cinerea	—
Fusarium oxysporum	—
Fusarium solani	—

TABLE 137.

Antifungal Activity of Salicylic Acid Derivatives

No.	Minimum inhibitory concentration for pathogenic fungi (Percent. W/V)								
	Candida species		Trichophyton species				Microsporum species		Epidermophyton
	albicans	krusei	verrucosum	mentagrophytes	rubrum	tonsurans	canis	audouini	floccosum
1	0.01 (11.8)	0.01 (12.0)	0.1 (12.5)	0.1 (15.1)	0.01 (16.1)	0.01 (18.3)	0.01 (20.0)	0.01 (19.5)	0.1 (15.4)
2	—	—	—	—	—	—	—	—	—
3	0.25 (11.5)	0.01 (11.8)	—	0.01 (20.5)	0.01 (15.9)	—	0.01 (17.8)	—	—
4	0.01 (13.2)	0.01 (11.0)	0.01 (25.0)	0.01 (18.5)	0.01 (20.0)	0.01 (19.9)	0.1 (15.6)	0.25 (20.1)	0.25 (18.1)
5	—	0.01 (15.5)	—	—	—	—	—	—	—
6	0.1 (10.5)	0.01 (10.5)	—	0.01 (22.5)	—	—	—	—	—
7	0.1 (13.5)	(0.1 (15.9)	—	—	0.1 (13.0)	—	—	—	—
8	0.1 (15.5)	0.01 (10.0)	—	0.01 (24.5)	0.25 (18.9)	—	—	—	—
9	0.1 (11.1)	0.1 (11.0)	—	0.01 (25.8)	—	—	0.1 (36.2)	—	—

Note—1. Figures in brackets indicate diameters of zones of inhibition in mm.

2. (—) indicates no activity.

Trypan Blue and other dyes/stains for protection against Newcastle disease virus (NDV) in chick embryos. An effective dose, 1 mg, of Trypan Blue was much less so if given after the virus injection instead of before. The effectiveness of two dosages of Trypan Blue are given in Table 138, also (in Column A) an indication that a preventive dose would have to be adjusted to the amount of infection expected.

TABLE #38.

Protective effect of Trypan blue against NDV in chick embryos

Log Virus Dilution	Mortality[a]						
	Control	Trypan blue[b]					
		A	B	C	D	E	F
−10	4/10						
−9	18/20						
−8	39/40	2/20	20/20	4/40	40/40	4/20	20/20
−7		6/19					
−6		17/20					
−5		19/19					

[a] No. dead/total, 92 hr after inoculation (data combined from two experiments).

[b] A, Trypan blue, lot 11003, 1000 μg/egg, immediately prior to virus; B, Trypan blue, lot 11003, 100 μg/egg, immediately prior to virus; C, Trypan blue, lot 16124, 1000 μg/egg, immediately prior to virus; D, Trypan blue, lot 16124, 100 μg/egg, immediately prior to virus; E, Trypan blue, lot 16124, 1000 μg/egg, 24 hr prior to virus; F, Trypan blue, lot 16124, 1000 μg/egg, 24 hr after virus.

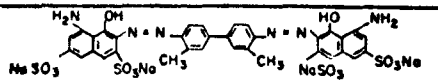
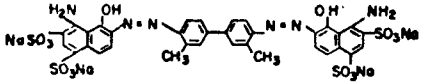
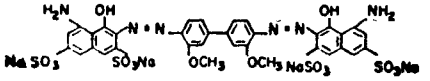
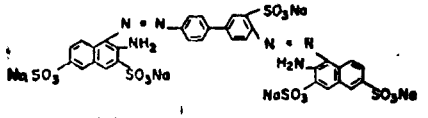
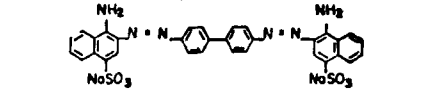
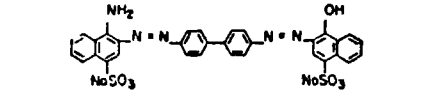
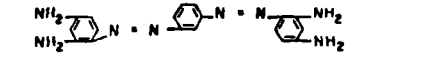
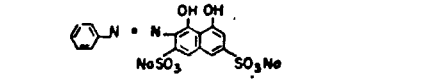
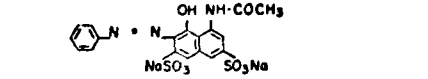
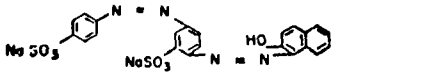
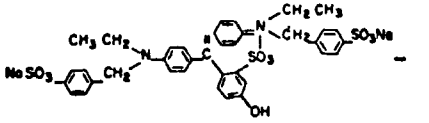
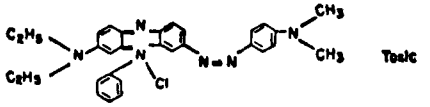
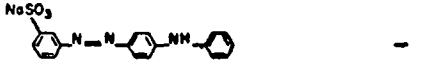
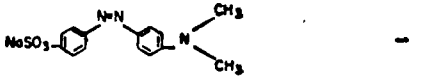
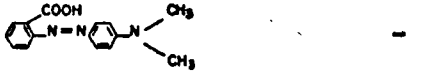
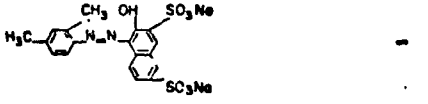
Table 139 presents the results of the other compounds tested, all in 1 mg doses.

Zsolnai (1964) tested many azo compounds against a variety of bacteria, with results in Tables 140 and 141, in units of minimum molar dilution still effective.

Zsolnai (1965) reported the results of some more azo compounds, in Tables 142 and 143.

TABLE 139

Effect of Trypan blue and other compounds on Newcastle disease virus in chick embryos and on hemagglutination

COMPOUND	STRUCTURAL FORMULA	PROTECTIVE*	INHIBITS HEMAGGLUTINATION
Trypan blue		+	+
Evans blue		+	+
Pontomine sky blue		+	+
Trypan Red		+	+
Congo Red		+	−
Erie garnet B		+	−
Bismarck brown Y		−	−
Chromotrope 2R		−	−
Fast crimson GH		−	−
Biebrich scarlet		−	−
Fast Green FCF		−	−
Janus green B		Toxic	−
Kernechtrot		−	−
Metanil yellow		−	−
Methyl Orange		−	−
Methyl red		−	−
Orange G		−	−
Ponceau 2R		−	±
Na-Anthraquinone-b-sulfonate		−	
Flavianic acid		−	

*1000 μg of compounds administered allantoically prior to challenge.

Table 140. Azo Compounds as Bacteriostats

Bacterium	Phenyl-azo-malo-nitril F/2201	2-Tolyl-azo-malo-nitril F 2202	3-Tolyl-azo-malo-nitril F/2203	4-Tolyl-azo-malo-nitril F/2204	2-Chlor-phenyl-azo-malo-nitril F/2205	3-Chlor-phenyl-azo-malo-nitril F/2206	4-Chlor-phenyl-azo-malo-nitril F/2207	4-Brom-phenyl-azo-malo-nitril F/2208	4-Jod-phenyl-azo-malo-nitril F/2209	4-Aethoxy-phenyl-azo-malo-nitril F/2210	2-Methyl-4-brom-phenyl-azo-malo-nitril F/2211
Staphylococcus aureus Duncan	M/10.000	M/10,000	M/25,000	M/10,000	M/25,000	M/50,000	M/50,000	M/50,000	M/50,000	M/25,000	M/25,000
Staphylococcus aureus pyogenes	M/10.000	M/10,000	M/10,000	M/10,000	M/25,000	M/50,000	M/50.000	M/50,000	M/50,000	M/10,000	M/25,000
Staphylococcus albus	M/10,000	M/10.000	M/10,000	M/10,000	M/25,000	M/50,000	M/50,000	M/50,000	M/50,000	M/10,000	M/25.000
Shigella dysenteriae Flexner	M/ 5.000	M/ 2.500	M/ 2,500	M/ 5,000	M/10,000	M/10,000	M/10,000	M/10,000	M/10,000	M/ 1,000	M/ 5.000
Shigella dysenteriae Sonne	M/ 5.000	M/ 2,500	M/ 2,500	M/ 2,500	M/10,000	M/10,000	M/10,000	M/10,000	M/10.000	M/ 1,000	M/ 5,000
Salmonella typhi	M/10.000	M/10.000	M/10.000	M/10,000	M/25,000	M/50,000	M/50,000	M/25,000	M/25.000	M/ 2,500	M/10.000
Salmonella paratyphi	M/ 5.000	M/10,000	M/10,000	M/ 5,000	M/10.000	M/10,000	M/10.000	M/25,000	M/10.000	M/ 1,000	M/ 5,000
Escherichia coli communis	M/ 2.500	M/ 1.000	M/ 1,000	M/ 2,500	M/ 2.500	M/10,000	M/10,000	M/10.000	M/10.000	M/ 1,000	M/ 5.000
Aerobacter aerogenes	M/ 2.500	M/ 1,000	M/ 1,000	M/ 2,500	M/ 5,000	M/10,000	M/10.000	M/10.000	M/10.000	M/ 1.000	M/ 5.000
Proteus vulgaris	M/ 2.500	M/ 1.000	M/ 1,000	M/ 2,500	M/ 5.000	M/10,000	M/10.000	M/10.000	M/10.000	—	M/ 5.000
Pseudomonas pyocyanea	—	—	—	—	M/ 1,000	M/ 2,500	M/ 2,500	M/ 2,500	M/ 2.500	—	M/ 2.500
Pseudomonas fluorescens	—	—	—	—	M/ 1.000	M/ 2,500	M/ 2.500	M/ 2,500	M/ 2.500	—	M/ 1.000

Bacterium	2-Methyl-4-jod-phenyl-azo-malo-nitril F 2212	3-Methyl-4-brom-phenyl-azo-malo-nitril F 2213	4-Methyl-2-brom-phenyl-azo-malo-nitril F/2214	2-Brom-4-aethoxy-phenyl-azo-malo-nitril F/2215	2,5-Di-chlor-phenyl-azo-malo-nitril F/2216	3,5-Di-brom-phenyl-azo-malo-nitril F/2217	2-Chlor-4-brom-phenyl-azo-malo-nitril F/2218	3-Chlor-4-brom phenyl-azo-malo-nitril F/2219	2-Methyl-4,6-di-brom-phenyl-azo-malo-nitril F 2220	2-Chlor-4,6-di-brom-phenyl-azo-malo-nitril F/2221	3-Chlor-4,6-di-brom-phenyl-azo-malo-nitril F/2222
Staphylococcus aureus Duncan	M/50.000	M/50.000	M/25,000	M/25,000	M/50,000	M/50,000	M/50,000	M/50,000	M/50,000	M/50,000	M/50.000
Staphylococcus aureus pyogenes	M/50.000	M/50.000	M/25,000	M/25,000	M/50,000	M/50,000	M/50,000	M/50,000	M/50,000	M/50,000	M/50,000
Staphylococcus albus	M/50.000	M/50.000	M/25.000	M/25,000	M/50,000	M/50,000	M/50,000	M/50,000	M/50.000	M/50,000	M/50,000
Shigella dysenteriae Flexner	M/ 5.000	M/ 5.000	M/ 2,500	M/ 1.000	M/ 5,000	M/25,000	M/10,000	M/10,000	M/ 2,500	M/ 2,500	M/ 5.000
Shigella dysenteriae Sonne	M/ 5.000	M/ 5.000	M/ 2,500	M/ 1,000	M/ 5.000	M/10.000	M/ 5.000	M/ 5.000	M/ 2.500	M/ 1,000	M/ 2,500
Salmonella typhi	M/10.000	M/10.000	M/10.000	M/ 5.000	M/10.000	M/25.000	M/25.000	M/25.000	M/10.000	M/10,000	M/10.000
Salmonella paratyphi	M/ 5.000	M/10.000	M/ 5.000	M/ 2,500	M/ 5.000	M/10.000	M/10.000	M/ 5.000	M/ 2.500	M/ 2,500	M/ 2,500
Escherichia coli communis	M/ 5.000	M/ 5.000	M/ 2,500	M/ 1.000	M/ 5.000	M/ 5.000	M/ 5.000	M/ 5.000	M/ 2.500	M/ 1,000	M/ 2,500
Aerobacter aerogenes	M/ 5.000	M/ 5.000	M/ 2,500	M/ 1.000	M/ 5,000	M/ 5.000	M/ 5.000	M/ 5.000	M/ 2.500	M/ 1,000	M/ 1.000
Proteus vulgaris	M/ 5.000	M/ 5.000	M/ 2,500	M/ 1,000	M/ 5.000	M/ 5.000	M/10.000	M/ 5.000	M/ 2.500	M/ 1.000	M/ 2.500
Pseudomonas pyocyanea	M/ 2.500	M/ 2.500	M/ 1.000	—	M/ 2.500	M/ 2,500	M/ 2.500	M/ 2.500	M/ 2.500	M/ 1.000	M/ 1,000
Pseudomonas fluorescens	M/ 2.500	M/ 2.500	M/ 1,000	—	M/ 2,500	M/ 2,500	M/ 2,500	M/ 2,500	M/ 2.500	M/ 1.000	M/ 1.000

Table 140. Continued

	4-Chlor-2.6-di-brom-phenyl-azo-malo-nitril F 2223	2,4,6-Tribrom-phenyl-azo-malo-nitril F/2224	2-Nitro-phenyl-azo-malo-nitril F/2225	3-Nitro-phenyl-azo-malo-nitril F/2226	4-Nitro-phenyl-azo-malo-nitril F 2227	2-Methyl-4-nitro-phenyl-azo-malo-nitril F/2228	3-Methyl-4-nitro-phenyl-azo-malo-nitril F/2229	4-Methyl-2-nitro-phenyl-azo-malo-nitril F 2230	2-Nitro-4-aethoxy-phenyl-azo-malo-nitril F 2231	2-Chlor-4-nitro-phenyl-azo-malo-nitril F 2232	3-Chlor-4-nitro-phenyl-azo-malo-nitril F 2233
Staphylococcus aureus Duncan	M 50,000	M 50,000	M/10,000	M/10,000	M/10.000	M/10.000	M/10,000	M 10,000	M 10,000	M 10.000	M 50.000
Staphylococcus aureus pyogenes	M 50.000	M 50.000	M/10,000	M/10,000	M/10,000	M/10,000	M/10,000	M 10.000	M 10,000	M 10.000	M 50,000
Staphylococcus albus	M 50,000	M 50.000	M/10,000	M/10,000	M/10.000	M/10,000	M/10,000	M 10,000	M 10,000	M 10,000	M 25.000
Shigella dysenteriae Flexner	M 2,500	M 2.500	M/ 5,000	M/ 5,000	M/ 5.000	—	M/ 1,000	M 2.500	M/ 1,000	M/ 2,500	M 5.000
Shigella dysenteriae Sonne	M 2.500	M 2.500	M/ 5,000	M/ 2,500	M/ 2.500	—	M/ 1,000	M 2.500	M/ 1.000	—	M 2.500
Salmonella typhi	M 5.000	M 5.000	M 25,000	M/10,000	M 10.000	M/ 5.000	M/ 5.000	M 5.000	M 2,500	M/ 2,500	M 10.000
Salmonella paratyphi	M 2.500	M 2.500	M/10,000	M/ 2,500	M/ 2.500	M/ 1,000	M/ 1,000	M 2.500	M 1.000	M/ 1.000	M 2.500
Escherichia coli communis	M 1,000	M/ 1,000	M/ 5,000	M/ 2,500	M 1.000	—	—	M 2.500	M 1,000	—	M 2.500
Aerobacter aerogenes	M 1,000	M/ 1.000	M/ 5,000	M/ 2,500	M/ 1,000	—	—	M 2.500	M/ 1,000	—	M 2,500
Proeteus vulgaris	M 2.500	M/ 1.000	M/ 5,000	M/ 2,500	M/ 1,000	—	—	M 1.000	M/ 1.000	—	M 2,500
Pseudomonas pyocyanea	M 1.000	M/ 1.000	—	—	—	—	—	—	—	—	M 1.000
Pseudomonas fluorescens	M/ 1,000	M/ 1,000	—	—	—	—	—	—	—	—	M 1,000

	4-Chlor-2-nitro-phenyl-azo-malo-nitril F 2234	Phenyl-azo-malo-nitril-4-karbon-säure-aethyl-ester F/2238	1-Naphtyl-azo-malo-nitril F/2243	4-Brom-1-naph-tyl-azo-malo-nitril F/2244	Azoben-zol-4-azo-malo-nitril F/2245	3,3'-Di-methyl-diphenyl-en-4,4'-bis-(azo-malo-nitril F/2247	Phenyl-azo-cyan-essig-säure methyl-ester F/2248	4-Tolyl-azo-cyan-essig-säure methyl-ester F/2249	4-Chlor-phenyl-azo-cyan-essig-saure-methyl-ester F/2250	Phenyl-azo-cyan-essig-saure-aethyl ester F 2251	4-Tolyl-azo-cyan-essig-säure-aethyl-ester F 2252
Staphylococcus aureus Duncan	M 50.000	M 25.000	M 50.000	M/50,000	M/50,000	M/10,000	—	—	—	—	—
Staphylococcus aureus pyogenes	M 50.000	M 25.000	M 50.000	M/50,000	M/50.000	M/10,000	—	—	—	—	—
Staphylococcus albus	M 50.000	M 25.000	M 50.000	M/50,000	M/50,000	M/10,000	—	—	—	—	—
Shigella dysenteriae Flexner	M 5.000	M 1.000	M 5.000	M/ 5,000	—	—	—	—	—	—	—
Shigella dysenteriae Sonne	M 2.500	M 1.000	M 2,500	M/ 2.500	—	—	—	—	—	—	—
Salmonella typhi	M 25.000	M 5.000	M 10.000	M/10,000	—	—	—	—	—	—	—
Salmonella paratyphi	M 5.000	M 2.500	M 5.000	M 5.000	—	—	—	—	—	—	—
Escherichia coli communis	M 5.000	M 2.500	M 2,500	M 2,500	—	—	—	—	—	—	—
Aerobacter aerogenes	M 2.500	M 2.500	M 2,500	M/ 2,500	—	—	—	—	—	—	—
Proteus vulgaris	M 2.500	M 2.500	M 2,500	M/ 5,000	—	—	—	—	—	—	—
Pseudomonas pyocyanea	—	—	M 1,000	M/ 1,000	—	—	—	—	—	—	—
Pseudomonas fluorescens	—	—	M 1,000	M/ 1,000	—	—	—	—	—	—	—

Table 140. Continued

	4-Chlor-phenyl-azo-cyan-essig-säure-aethyl-ester F 2253	Phenyl-azo-cyan-essig-säure-anilid F/2257	4-Chlor-phenyl-azo-cyan-essig-säure-anilid F/2259	Phenyl-azo-cyan-essig-säure-(4'-chlor-anilid) F/2260	4-Chlor-phenyl-azo-cyan-essig-säure-(4'-chlor-anilid) F/2262	Phenyl-azo-cyan-essig-säure-hydrazid F/2263	4-Tolyl-azo-cyan-essig-säure-hydrazid F/2264	4-Chlor-phenyl-azo-cyan-essig-säure-hydrazid F/2265	Phenyl-azo-acetyl-aceton F 2266	3-Tolyl-azo-acetyl-aceton F 2267
Staphylococcus aureus Duncan	—	—	—	—	—	—	—	M/ 5,000	—	—
Staphylococcus aureus pyogenes	—	—	—	—	—	—	—	M/ 5,000	—	—
Staphylococcus albus	—	—	—	—	—	—	—	M/ 5,000	—	—
Shigella dysenteriae Flexner	—	—	—	—	—	—	—	—	—	—
Shigella dysenteriae Sonne	—	—	—	—	—	—	—	—	—	—
Salmonella typhi	—	—	—	—	—	—	—	—	—	—
Salmonella paratyphi	—	—	—	—	—	—	—	—	—	—
Escherichia coli communis	—	—	—	—	—	—	—	—	—	—
Aerobacter aerogenes	—	—	—	—	—	—	—	—	—	—
Proteus vulgaris	—	—	—	—	—	—	—	—	—	—
Pseudomonas pyocyanea	—	—	—	—	—	—	—	—	—	—
Pseudomonas fluorescens	—	—	—	—	—	—	—	—	—	—

	Phenyl-azo-acet-essig-ester F/2271	3-Tolyl-azo-acet-essig-ester F/2272	4-Tolyl-azo-acet-essig-ester F/2273	3-Chlor-phenyl-azo-acet-essig-ester F/2274	4-Chlor-phenyl-azo-acet-essig-ester F/2275	Phenyl-azo-malon-saure diaethyl-ester F/2276	4-Tolyl-azo-malon-säure-diaethyl-ester F/2277	4-Chlor-phenyl-azo-malonsaure diaethyl-ester F 2278
Staphylococcus aureus Duncan	—	—	—	—	—	—	—	—
Staphylococcus aureus pyogenes	—	—	—	—	—	—	—	—
Staphylococcus albus	—	—	—	—	—	—	—	—
Shigella dysenteriae Flexner	—	—	—	—	—	—	—	—
Shigella dysenteriae Sonne	—	—	—	—	—	—	—	—
Salmonella typhi	—	—	—	—	—	—	—	—
Salmonella paratyphi	—	—	—	—	—	—	—	—
Escherichia coli communis	—	—	—	—	—	—	—	—
Aerobacter aerogenes	—	—	—	—	—	—	—	—
Proteus vulgaris	—	—	—	—	—	—	—	—
Pseudomonas pyocyanea	—	—	—	—	—	—	—	—
Pseudomonas fluorescens	—	—	—	—	—	—	—	—

Table 141. Azo Compounds as Tuberculostats

1. Aryl-azo-malonitrile

F/2201	Phenyl-azo-malonitril	M/10,000
F/2202	2-Tolyl-azo-malonitril	M/ 5,000
F/2203	3-Tolyl-azo-malonitril	M/25,000
F/2204	4-Tolyl-azo-malonitril	M/25,000
F/2205	2-Chlor-phenyl-azo-malonitril	M/ 5,000
F/2206	3-Chlor-phenyl-azo-malonitril	M/50,000
F/2207	4-Chlor-phenyl-azo-malonitril	M/50,000
F/2208	4-Brom-phenyl-azo-malonitril	M/50,000
F/2209	4-Jod-phenyl-azo-malonitril	M/50,000
F/2210	4-Aethoxy-phenyl-azo-malonitril	M/25,000
F/2211	2-Methyl-4-brom-phenyl-azo-malonitril	M/25,000
F/2212	2-Methyl-4-jod-phenyl-azo-malonitril	M/25,000
F/2213	3-Methyl-4-brom-phenyl-azo-malonitril	M/50,000
F/2214	4-Methyl-2-brom-phenyl-azo-malonitril	M/10,000
F/2215	2-Brom-4-aethoxy-phenyl-azo-malonitril	M/50,000
F/2216	2,5-Dichlor-phenyl-azo-malonitril	M/50,000
F/2217	3,5-Dibrom-phenyl-azo-malonitril	M/50,000
F/2218	2-Chlor-4-brom-phenyl-azo-malonitril	M/25,000
F/2219	3-Chlor-4-brom-phenyl-azo-malonitril	M/50,000
F/2220	2-Methyl-4,6-dibrom-phenyl-azo-malonitril	M/25,000
F/2221	2-Chlor-4,6-dibrom-phenyl-azo-malonitril	M/25,000
F/2222	3-Chor-4,6-dibrom-phenyl-azo-malonitril	M/25,000
F/2223	4-Chor-2,6-dibrom-phenyl-azo-malonitril	M/25,000
F/2224	2,4,6-Tribrom-phenyl-azo-malonitril	M/10,000
F/2225	2-Nitro-phenyl-azo-malonitril	M/10,000
F/2226	3-Nitro-phenyl-azo-malonitril	M/25,000
F/2227	4-Nitro-phenyl-azo-malonitril	M/25,000
F/2228	2-Methyl-4-nitro-phenyl-azo-malonitril	M/10,000
F/2229	3-Methyl-4-nitro-phenyl-azo-malonitril	M/25,000
F/2230	4-Methyl-2-nitro-phenyl-azo-malonitril	M/10,000
F/2231	2-Nitro-4-aethoxy-phenyl-azo-malonitril	M/10,000
F/2232	2-Chlor-4-nitro-phenyl-azo-malonitril	M/25,000
F/2233	3-Chlor-4-nitro-phenyl-azo-malonitril	M/25,000
F/2234	4-Chlor-2-nitro-phenyl-azo-malonitril	M/25,000
F/2235	4-Acetylamino-phenyl-azo-malonitril	M/10,000
F/2236	Phenyl-azo-malonitril-2-karbonsäure	—
F/2237	Phenyl-azo-malonitril-4-karbonsäure	—
F/2238	Phenyl-azo-malonitril-4-karbonsäure-aethyl-ester	M/25,000
F/2239	3-Hydroxy-phenyl-azo-malonitril-4-karbonsäure	M/ 2,500
F/2240	Phenyl-azo-malonitril-4-sulfonsäure	—
F/2241	Phenyl-azo-malonitril-4-sulfonamid	—
F/2242	Phenyl-azo-malonitril-4-N-(4',6'-dimethyl-2-pyrimidyl)-sulfonamid	—
F/2243	1-Naphtyl-azo-malonitril	M/25,000
F/2244	4-Brom-1-naphtyl-azo-malonitril	M/50,000
F/2245	Azobenzol-4-azo-malonitril	M/50,000
F/2246	Diphenylen-4,4'-bis(azo-malonitril)	M/ 5,000
F/2247	3,3'-Dimethyl-diphenylen-4,4'-bis-(azo-malonitril)	M/ 5,000

2. Aryl-azo-cyanessigsäure-ester

F/2248	Phenyl-azo-cyanessigsäure-methyl-ester	M/ 2,500
F/2249	4-Tolyl-azo-cyanessigsäure-methyl-ester	M/ 2,500
F/2250	4-Chlor-phenyl-azo-cyanessigsäure-methyl-ester	M/ 2,500
F/2251	Phenyl-azo-cyanessigsäure-aethyl-ester	M/ 5,000
F/2252	4-Tolyl-azo-cyanessigsäure-aethyl-ester	M/ 5,000
F/2253	4-Chlor-phenyl-azo-cyanessigsäure-aethyl-ester	M/ 5,000

3. Aryl-azo-cyanacetamide und ihre N-substituierte Derivate

F/2254	Phenyl-azo-cyanacetamid	—
F/2255	4-Tolyl-azo-cyanacetamid	—
F/2256	4-Chlor-phenyl-azo-cyanacetamid	—
F/2257	Phenyl-azo-cyanessigsäure-anilid	M/ 1,000
F/2258	4-Tolyl-azo-cyanessigsäure-anilid	M/ 1,000
F/2259	4-Chlor-phenyl-azo-cyanessigsäure-anilid	M/ 1,000
F/2260	Phenyl-azo-cyanessigsäure-(4'-chlor-anilid)	M/ 2,500
F/2261	4-Tolyl-azo-cyanessigsäure-(4'-chlor-anilid)	M/ 5,000
F/2262	4-Chlor-phenyl-azo-cyanessigsäure-(4'-chlor-anilid)	M/ 5,000
F/2263	Phenyl-azo-cyanessigsäure-hydrazid	M/10,000
F/2264	4-Tolyl-azo-cyanessigsäure-hydrazid	M/10,000
F/2265	4-Chlor-phenyl-azo-cyanessigsäure-hydrazid	M/10,000

4. Aryl-azo-Derivate des Acetylacetons, Acetessigesters und Malonsäure-diaethyl-esters

F/2266	Phenyl-azo-acetylaceton	M/ 2,500
F/2267	3-Tolyl-azo-acetylaceton	M/ 2,500
F/2268	4-Tolyl-azo-acetylaceton	M/ 2,500
F/2269	3-Chlor-phenyl-azo-acetylaceton	M/ 2,500
F/2270	4-Chlor-phenyl-azo-acetylaceton	M/ 2,500
F/2271	Phenyl-azo-acetessigester	M/10,000
F/2272	3-Tolyl-azo-acetessigester	M/10,000
F/2273	4-Tolyl-azo-acetessigester	M/10,000
F/2274	3-Chlor-phenyl-azo-acetessigester	M/10,000
F/2275	4-Chlor-phenyl-azo-acetessigester	M/10,000
F/2276	Phenyl-azo-malonsäure-diaethyl-ester	M/ 5,000
F/2277	4-Tolyl-azo-malonsäure-diaethyl-ester	M/ 5,000
F/2278	4-Chlor-phenyl-azo-malonsäure-diaethyl-ester	M/ 2,500

Table 142. Azo Compounds as Bacteriostats

Bacterium	4-Acetyl-phenyl-azo-malonitril F/2313	4-Acetyl-phenyl-azo-malonitril-semicarbazon F/2318	4-Acetyl-phenyl-azo-malonitril-oxym F/2321	4-Acetyl-phenyl-azo-malonitril-phenyl-hydrazon F/2322	4-Acetyl-phenyl-azo-malonitril-salizyloyl-hydrazon F/2323	4-Acetyl-phenyl-azo-malonitril-isonikotinoyl-hydrazon F/2324	4-Acetyl-phenyl-azo-cyanessigsäure-methyl-ester-amino-guanidon hydrochlorid F/2328	4-(4'-Acetyl-phenyl-azo)-3,5-dimethyl-pyrazolyl-1-amidin-aminoguanidon dihydrochlorid F/2330
Staphylococcus aureus Duncan	M/10,000	M/ 2,500	M/ 1 000	M/10,000	M/ 5,000	M/ 2,500	M/ 2,500	M/10,000
Staphylococcus aureus pyogenes	M/10,000	M/ 2,500	M/ 1,000	M/10,000	M/ 5,000	M/ 2,500	M/ 2,500	M/10,000
Staphylococcus albus	M/ 5,000	M/ 2,500	(M/1000)	M/ 5,000	M/ 5,000	(M/1000)	M/ 1,000	M/10,000
Shigella dysenteriae Flexner	M/ 5,000	M/ 2,500	M/ 1,000	M/ 5,000	M/ 2,500	M/ 1,000	M/ 1,000	—
Shigella dysenteriae Sonne	M/ 5,000	M/ 1,000	—	M/ 2,500	M/ 2,500	—	—	—
Salmonella typhi	M/10,000	M/ 2,500	—	M/ 5,000	M/ 2,500	—	—	—
Salmonella paratyphi	M/ 5,000	M/ 1,000	—	M/ 1,000	—	—	—	—
Escherichia coli communis	M/ 1,000	M/ 1,000	—	M/ 1,000	—	—	—	—
Aerobacter aerogenes	M/ 1,000	—	—	M/ 1,000	—	—	—	—
Proteus vulgaris	M/ 1,000	—	—	M/ 1,000	—	—	—	—
Pseudomonas pyocyanea	—	—	—	—	—	—	—	—
Pseudomonas fluorescens	—	—	—	—	—	—	—	—

Bacterium	4-(4'-Acetyl-phenyl-azo)-3-methyl-pyrazolon(5)-yl-1-amidin-amino-guanidon dihydrochlorid F/2331	4-Phenyl-azo-3,5-dimethyl-pyrazolyl-1-amidin hydrochlorid F/2344	4-(3'-Tolyl-azo)-3,5-dimethyl-pyrazolyl-1-amidin hydrochlorid F/2345	4-(4'-Tolyl-azo)-3,5-dimethyl-pyrazolyl-1-amidin hydrochlorid F/2346	4-(3'-Chlor-phenyl-azo)-3,5-dimethyl-pyrazolyl-1-amidin hydrochlorid F/2347	4-(4'-Chlor-phenyl-azo)-3,5-dimethyl-pyrazolyl-1-amidin hydrochlorid F/2348	4-(4'-Tolyl-azo)-3,5-dimethyl-pyrazolyl-1-thiocarbon-säureamid F/2356	4-(3'-Chlor-phenyl-azo)-3,5-dimethyl-pyrazolyl-1-thiocarbon-säureamid F/2357
Staphylococcus aureus Duncan	M/ 5,000	M/ 5,000	M/ 5,000	M/10,000	M/10,000	M/10,000	M/ 2,500	M/ 2,500
Staphylococcus aureus pyogenes	M/ 5,000	M/ 2,500	M/ 5,000	M/10,000	M/10 000	M/10,000	M/ 2,500	M/ 2,500
Staphylococcus albus	M/ 2,500	M/ 5,000	M/ 5,000	M/10,000	M/10,000	M/10,000	M/ 2,500	M/ 2,500
Shigella dysenteriae Flexner	—	M/ 2,500	M/ 2,500	M/ 2,500	M/ 2,500	M/ 2,500	—	—
Shigella dysenteriae Sonne	—	M/ 1,000	M/ 1,000	M/ 1,000	(M/1000)	M/ 1,000	—	—
Salmonella typhi	—	M/ 1,000	M/ 1,000	M/ 1,000	(M/1000)	M/ 1,000	—	—
Salmonella paratyphi	—	M/ 1,000	M/ 1,000	M/ 1,000	(M/1000)	M/ 1,000	—	—
Escherichia colicommunis	—	M/ 1,000	M/ 1,000	M/ 1,000	—	M/ 1,000	—	—
Aerobacter aerogenes	—	—	—	—	—	—	—	—
Proteus vulgaris	—	—	—	—	—	—	—	—
Pseudomonas pyocyanea	—	—	—	—	—	—	—	—
Pseudomonas fluorescens	—	—	—	—	—	—	—	—

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Table 142. Continued

	4-(4'-Chlor-phenyl-azo)-3,5-dimethyl-pyrazolyl-thiocarbon-säureamid F/2358	4-Chlor-benzal-malonitril F/2371	Benzyl-brom-malonitril F/2398	4-Chlor-benzyl-brom-malonitril F/2399	Benzyl-malonitril (als Ausgangsstoff)	4-Chlor-benzyl-malonitril (als Ausgangsstoff)	Phenyl-malonitril (als Ausgangsstoff)
Staphylococcus aureus Duncan	M/ 2,500	—	—	M/ 1,000	—	—	M/ 1,000
Staphylococcus aureus pyogenes	M/ 2,500	—	—	M/ 1,000	—	—	M/ 1,000
Staphylococcus albus	M/ 2,500	—	—	M/ 1,000	—	—	M/ 1,000
Shigella dysenteriae Flexner	—	—	—	M/ 1,000	—	—	M/ 2,500
Shigella dysenteriae Sonne	—	—	—	M/ 1,000	—	—	M/ 2,500
Salmonella typhi	—	—	—	M/ 1,000	—	—	M/ 2,500
Salmonella paratyphi	—	—	—	—	—	—	M/ 2,500
Escherichia coli communis	—	—	—	—	—	—	M/ 2,500
Aerobacter aerogenes	—	—	—	—	—	—	M/ 1,000
Proteus vulgaris	—	—	—	—	—	—	M/ 1,000
Pseudomonas pyocyanea	—	—	—	—	—	—	M/ 1,000
Pseudomonas fluorescens	—	—	—	—	—	—	M/ 1,000

Table 143. Azo Compounds as Tuberculostats

1. Aryl-azo-Derivate von Alkyl- und Aralkyl-malonitrilen		
F/2279	Phenyl-azo-methyl-malonitril	M/ 2,500
F/2280	2-Tolyl-azo-methyl-malonitril	M/ 2,500
F/2281	3-Tolyl-azo-methyl-malonitril	M/ 5,000
F/2282	4-Tolyl-azo-methyl-malonitril	M/ 5,000
F/2283	2-Chlor-phenyl-azo-methyl-malonitril	M/ 2,500
F/2284	3-Chlor-phenyl-azo-methyl-malonitril	M/ 5,000
F/2885	4-Chlor-phenyl-azo-methyl-malonitril	M/ 5,000
F/2286	4-Aethoxy-phenyl-azo-methyl-malonitril	M/10,000
F/2287	Phenyl-azo-aethyl-malonitril	M/ 5,000
F/2288	2-Tolyl-azo-aethyl-malonitril	M/ 5,000
F/2289	3-Tolyl-azo-aethyl-malonitril	M/ 5,000
F/2290	4-Tolyl-azo-aethyl-malonitril	M/ 5,000
F/2291	2-Chlor-phenyl-azo-aethyl-malonitril	M/ 2,500
F/2292	3-Chlor-phenyl-azo-aethyl-malonitril	M/ 5,000
F/2293	4-Chlor-phenyl-azo-aethyl-malonitril	M/ 5,000
F/2294	4-Aethoxy-phenyl-azo-aethyl-malonitril	M/10,000
F/2295	Phenyl-azo-benzyl-malonitril	M/ 5,000
F/2296	2-Tolyl-azo-benzyl-malonitril	M/ 5,000
F/2297	3-Tolyl-azo-benzyl-malonitril	M/10,000
F/2298	4-Tolyl-azo-benzyl-malonitril	M/10,000
F/2299	2-Chlor-phenyl-azo-benzyl-malonitril	M/10,000
F/2300	3-Chlor-phenyl-azo-benzyl-malonitril	M/10,000
F/2301	4-Chlor-phenyl-azo-benzyl-malonitril	M/10,000
F/2302	Phenyl-azo-(4'-chlor-benzyl)-malonitril	M/10,000
F/2303	3-Tolyl-azo-(4'-chlor-benzyl)-malonitril	M/25,000
F/2304	4-Tolyl-azo-(4'-chlor-benzyl)-malonitril	M/25,000
F/2305	2-Chlor-phenyl-azo-(4'-chlor-benzyl)-malonitril	M/10,000
F/2306	3-Chlor-phenyl-azo-(4'-chlor-benzyl)- malonitril	M/10,000
F/2307	4-Chlor-phenyl-azo-(4'-chlor-benzyl)-malonitril	M/10,000
2. Aryl-azo-benzoyl-acetone		
F/2308	Phenyl-azo-benzoyl-aceton	M/ 5,000
F/2309	3-Tolyl-azo-benzoyl-aceton	M/ 5,000
F/2310	4-Tolyl-azo-benzoyl-aceton	M/ 5,000
F/2311	3-Chlor-phenyl-azo-benzoyl-aceton	M/ 5,000
F/2312	4-Chlor-phenyl-azo-benzoyl-aceton	M/10,000
3. 4-Acetyl-phenyl-azo-Derivate von "aktive Methylen-Gruppe" enthaltenden Verbindungen.		
F/2313	4-Acetyl-phenyl-azo-malonitril	M/25,000
F/2314	4-Acetyl-phenyl-azo-cyanessigsäure-methyl-ester	M/ 1,000
F/2315	4-Acetyl-phenyl-azo-cyanacetamid	—
F/2316	4-Acetyl-phenyl-azo-acetylaceton	M/ 1,000
F/2317	4-Acetyl-phenyl-azo-acetessigester	M/ 2,500
4. Mit Karbonyl-Reagentien gebildete Derivate von 4-Acetyl-phenyl-azo-methylen-Gruppe enthaltenden Verbindungen		
F/2318	4-Acetyl-phenyl-azo-malonitril-semicarbon	M/25,000
F/2319	4-Acetyl-phenyl-azo-malonitril-thiosemicarbazon	M/25,000
F/2320	4-Acetyl-phenyl-azo-malonitril-aminoguanidon hydrochlorid	M/10,000
F/2321	4-Acetyl-phenyl-azo-malonitril-oxym	M/ 5,000
F/2322	4-Acetyl-phenyl-azo-malonitril-phenyl-hydrazon	M/25,000
F/2323	4-Acetyl-phenyl-azo-malonitril-salizyloyl-hydrazon	M/10,000
F/2324	4-Acetyl-phenyl-azo-malonitril-isonikotinoyl-hydrazon	M/1,250,000
F/2325	4-Acetyl-phenyl-azo-malonitril-azin	M/10,000
F/2326	4-Acetyl-phenyl-azo-cyanessigsäure-methyl-ester-semicarbazon	M/25,000
F/2327	4-Acetyl-phenyl-azo-cyanessigsäure-methyl-ester-thiosemicarbazon	M/ 5,000
F/2328	4-Acetyl-phenyl-azo-cyanessigsäure-methyl-ester-aminoguanidon hydrochlorid	M/10,000
F/2329	4-(4'-Acetyl-phenyl-azo)-3,5dimethyl-pyrazolyl-1-thiocarbonsäureamid-thiosemicarbazon	M/100,000
F/2330	4-(4'-Acetyl-phenyl-azo)-3,5-dimethyl-pyrazolyl-1-amidinaminoguanidon dihydrochlorid	M/25,000
F/2331	4-(4'-Acetyl-phenyl-azo)-3-methyl-pyrazolon-(5)-yl-1-amidinaminoguanidon dihydrochlorid	M/10,000

Table 143. Continued

5. Mit Hydrazin gebildete Derivate von Aryl-azo-methylen-Gruppe enthaltenden Verbindungen

F/2332	4-Phenyl-azo-3,5-diamino-pyrazol	M/ 2,500
F/2333	4-(4'-Tolyl-azo)-3,5-diamino-pyrazol	M/ 5,000
F/2334	4-(4'-Chlor-phenyl-azo-)3,5-diamino-pyrazol	M/10,000
F/2335	4-Phenyl-azo-3,5-dimethyl-pyrazol	M/10,000
F/2336	4-(4'-Tolyl-azo)-3,5-dimethyl-pyrazol	M/10,000
F/2337	4-(4'-Chlor-phenyl-azo)-3,5-dimethyl-pyrazol	M/25,000
F/2338	4-Phenyl-azo-3-amino-pyrazolon-(5)	M/10,000
F/2339	4-(4'-Tolyl-azo)-3-amino-pyrazolon-(5)	M/10,000
F/2340	4-(4'-Chlor-phenyl-azo)-3-amino-pyrazolon-(5)	M/10,000
F/2341	4-Phenyl-azo-3-methyl-pyrazolon-(5)	M/ 5,000
F/2342	4-(4'-Tolyl-azo)-3-methyl-pyrazolon-(5)	M/10,000
F/2343	4-(4'-Chlor-phenyl-azo)-3-methyl-pyrazolon-(5)	M/ 5,000

6. Mit Aminoguanidin-hydrochlorid gebildete Kondensationsprodukte von Aryl-azo-acetylacetonen und Aryl-azo-acetessigestern.

F/2344	4-Phenyl-azo-3,5-dimethyl-pyrazolyl-1-amidin hydrochlorid	M/ 5,000
F/2345	4-(3'-Tolyl-azo)-3,5-dimethyl-1-amidin hydrochlorid	M/ 5,000
F/2346	4-(4'-Tolyl-azo)-3,5-dimethyl-pyrazolyl-1-amidin hydrochlorid	M/ 5,000
F/2347	4-(3'-Chlor-phenyl-azo)-3,5-dimethyl-pyrazolyl-1-amidin hydrochlorid	M/ 5,000
F/2348	4-(4'-Chlor-phenyl-azo)-3,5-dimethyl-pyrazolyl-1-amidin hydrochlorid	M/ 5,000
F/2349	4-Phenyl-azo-3-methyl-pyrazolon-(5)-yl-1-amidin hydrochlorid	M/ 2,500
F/2350	4-(3'-Tolyli-azo)-3-methyl-pyrazolon-(5)-yl-1-amidin hydrochlorid	M/ 2,500
F/2351	4-(4'-Tolyl-azo)-3-methyl-pyrazolon-(5)-yl-1-amidin hydrochlorid	M/ 2,500
F/2352	4-(3'-Chlor-phenyl-azo)-3-methyl-pyrazolon-(5)-yl-1-amidin hydrochlorid	M/ 2,500
F/2353	4-(4'-Chlor-phenyl-azo)-3-methyl-pyrazolon-(5)-yl-1-amidin hydrochlorid	M/ 2,500

7. Mit Thiosemicarbazid gebildete Kondensationsprodukte von Aryl-azo-acetylacetonen.

F/2354	4-Phenyl-azo-3,5-dimethyl-pyrazolyl-1-thiocarbonsäureamid	M/10,000
F/2355	4-(3'-Tolyl-azo)-3,5-dimethyl-pyrazolyl-1-thiocarbonsäureamid	M/10,000
F/2356	4-(4'-Tolyl-azo)-3,5-dimethyl-pyrazolyl-1-thiocarbonsäureamid	M/10,000
F/2357	4-(4'-Chlor-phenyl-azo)-3,5-dimethyl-pyrazolyl-1-thiocarbonsäureamid	M/10,000
F/2558	4-(4'-Chlor-phenyl-azo)-3,5-dimethyl-pyrazolyl-o-thiocarbonsäureamid	M/10,000

8. Verschiedene andere Azo-Verbindungen

F/2359	4-Tolyl-azo-nitromethan (=Nitro-formaldehyd-4-tolyl-hydrazon)	M/10,000
F/2360	4-Chlor-phenyl-azo-nitromethan (=Nitro-formaldehyd-4-chlor-phenyl-hydrazon)	M/ 5,000
F/2361	Phenyl-azo-dicyandiamin	M/ 2,500
F/2362	2-Tolyl-azo-dicyandiamin	M/ 2,500
F/2363	3-Tolyl-azo-dicyandiamin	M/ 2,500
F/2364	4-Tolyl-azo-dicyandiamin	M/ 2,500
F/2365	2-Chlor-phenyl-azo-dicyandiamin	M/ 1,000
F/2366	3-Chlor-phenyl-azo-dicyandiamin	M/ 2,500
F/2367	4-Chlor-phenyl-azo-dicyandiamin	M/ 2,500
F/2368	2-Aethoxy-phenyl-azo-dicyandiamin	M/ 1,000
F/2369	4-Aethoxy-phenyl-azo-dicyandiamin	M/10,000

Malyuga et al (1971) reported on the antitubercular activity of some phenylazo-5-salicylic acid and 2-carboxy-4-phenylazonaphthol-1 derivatives, the substituents being on the "phenyl" ring. In order of decreasing effectiveness in the salicylic acid series were: 4-chloro, 4-bromo = 4-iodo = 4-methoxy = 4-carbethoxy, 2-, 3-, or 4-methyl, 3-nitro = no substituent, 4-nitro, 2-nitro. This order in the naphthol series was: 4-methyl, 4-methoxy, 2-nitro = 4-carbethoxy = 4-iodo = 4-chloro, 3-methyl, no substituent, 4-bromo, 4-nitro = 3-nitro.

XI. CURRENT REGULATIONS

There is only one non-dye azo compound for which a use regulation has been set, azodicarbonamide, 45 ppm in flour. Section III dealt with world-wide practices regarding food, drug, and cosmetic use of azo dyes.

XII. STANDARDS

No information was found.

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APPENDIX

A list of synonyms and tradenames follows for those azo compounds most frequently seen in the literature--for whatever reason. In the case of dyes the "entry" name in the alphabetization was occasionally somewhat arbitary. The source of azobisisobutyronitrile and azodicarbonamide was Modern Plastics Encyclopedia 1972-73, Vol. 49: No. 10A, p. 293 (1972). Two sources were used for the remaining entries: Colour Index, 2nd edition, and Desktop Analysis Tool for the Common Data Base (1968).

Amaranth

$N_2O_{10}S_3C_{20}H_{14}.3Na$

1302 Red CI
1508 Red CI
C.I. 16185 CI
Acid Amaranth CI
Acid Amaranth I CI
Acid Amaranth N CI
Acid Leather Red 12BW CI
Acid Leather Rubine S CI
Aizen Amaranth CI
Amacid Amaranth CI
Amaranth MERCK,CI,LC,NF-A,USP,USP-A
Amaranth A CI
Amaranth B CI
Amaranth B.P.C. CI
Amaranth S CI
Amaranth (the dye)
Amaranth BPC CI
Amaranthe CI
Amaranth Extra CI
Amaranth Lake CI
Amaranth S Specially Pure CI
Amaranth USP CI
Amaranth WD CI
Azo Red R CI
Azo Rubine S
S-Azo Rubine
Azo Rubine S.FQ CI
Azo Rubine SF CI
Azo Ruby S
Bordeaux S Extra Conc. A.Export CI
Bordeaux S Extra Pure A CI
Canacert Amaranth CI
Certicol Amaranth S CI
Cilefa Rubine 2B CI
C.I. Acid Red 27 CI
C.I. Acid Red 27, trisodium salt
C.I. Food Red 9 CI
Daishiki Amaranth CI
Dolkwal Amaranth CI
Dye FDC red 2 CARF

Amaranth cont.

Edicol Supra Amaranth A CI
Eurocert Amaranth CI
FD and C Red No. 2 MERCK,CI,LC
FD and C Red No. 2-Aluminum Lake CI
Food Red 2 CI
Fruit Red A Geigy CI
HD Amaranth B CI
HD Amaranth Supra CI
Hexacert Red No. 2 CI
Hexacol Amaranth B Extra CI
Hidacid Amaranth CI
Hispacid Red AM CI
2-Hydroxy-1,1'-azonaphthalene-3,6,4'-trisulfonic acid trisodium salt
Java Amaranth CI
Kayaku Amaranth CI
Kayaku Food Colour Red No. 2 CI
KCA Foodcol Amaranth A CI
Kiton Rubine S CI
Lissamine Amaranth AC CI
Maple Amaranth CI
2,7-Naphthalenedisulfonic acid, 3-hydroxy-4-[(4-sulfo-1-naphthyl)azo] -, trisodium salt
Naphthol Red B CI
Naphthol Red S CI
Naphthol Red S Conc. Specially Pure CI
Naphthol Red LZS CI
Naphthol Red SI CI
Naphthol Red S Specially Pure CI
Neklacid Red A CI
Rakuto Amaranth CI
Raspberry Red for Jellies CI
San-ei Amaranth CI
Shikiso Amaranth CI
Solar Red D CI
1-(4-Sulfo-1-naphthylazo)-2-naphthol-3,6-disulfonic acid trisodium salt CARF
Takaoka Amaranth CI
Tertracid Red A CI
Toyo Amaranth CI
Trisodium salt of 1-(4-sulfo-1-naphthylazo)-2-naphthol-3,6-disulfonic acid MERCK
Usacert Red No. 2 CI
Victoria Rubine O CI
Victoria Rubine O for Food CI
Whortleberry Red CI
Wool Bordeaux 6RK CI
Wool Red 40F CI

Aminoazobenzene

$N_2C_{12}H_{11}$
C.I. 11000 CI
Aminoazobenzene CI
p-Aminoazobenzene MERCK
4-Aminoazobenzene
Aminoazobenzene (Indicator) CI
p-Aminoazobenzol
4-Aminoazobenzol
p-Aminodiphenylimide
Aniline, p-(phenylazo)- CI
Aniline Yellow CI
Azobenzene, 4-amino-
4-Benzeneazoaniline
Brasilazina Oil Yellow G CI
Cellitazol R CI
Ceres Yellow R CI
C.I. Solvent Blue 7 CI
C.I. Solvent Yellow 1 CI
Fast Spirit Yellow CI
Fat Yellow AAB CI
Induline R CI
Oil Yellow AAB CI
Oil Yellow AB CI
Oil Yellow AN CI
Organol Yellow 2A CI
p-(Phenylazo)aniline
p-Phenylazophenylamine
Somalia Yellow 2G CI
Sudan Yellow R CI

o-Aminoazotoluene

$C_{14}H_{15}N_3$

C.I. 11160 CI
C.I. 11160B CI
Aminoazotoluene CI
o-Aminoazotoluene MERCK
2-Amino-5-azotoluene
4-Amino-2',3-dimethylazobenzene
o-AT
Brasilazina Oil Yellow R CI
C.I. Solvent Yellow 3 CI
2',3-Dimethyl-4-aminoazobenzene
Fast Garnet GBC base
Fast Yellow AT CI
Fat Yellow B CI
Hidaco Oil Yellow CI
OAAT
Oil Yellow CI
Oil Yellow C CI
Oil Yellow I CI
Oil Yellow 2R CI
Oil Yellow 21 CI
Oil Yellow 2681 CI
Oil Yellow AT CI
Organol Yellow 2T CI
Somalia Yellow R CI
Sudan Yellow RRA CI
Toluazotoluidine
o-Toluidine, 4-(o-tolylazo)- CI
4-(o-Tolylazo)-o-toluidine
Tulabase Fast Garnet GB CI
Tulabase Fast Garnet GBC CI

C.I. Solvent Yellow 3

HUE Yellow

Aminoazotoluene ... ...

Aminoazotoluene (*indicator*) ...
Brasilazina Oil Yellow R ...
Fast Oil Yellow ... ...
Fast Spirit Yellow ... ...
Fat Yellow B ... ...
Fast Yellow AT ... ...
Oil Yellow ... ...
Oil Yellow I ... ...
Oil Yellow 21 ... ...
Oil Yellow 2681 ... ...
Oil Yellow AT ... ...
Oil Yellow C ... ...
Oil Yellow 2R ... ...
Oil Yellow T ... ...
Oil Yellow T ... ...
Organol Yellow 2T ... ...
Somalia Yellow R ... ...
Sudan Yellow RRA ... ...
Waxakol Yellow NL ... ...

Azobenzene

$N_2C_{12}H_{10}$

Azobenzene MERCK,PI,FCH,GCUCP
Azobenzide GCUCP,PI
Azobenzol PI,MERCK
Benzene, azodi-
Benzeneazobenzene MERCK
Diphenyldiimide PI,GCUCP

Azobisisobutyronitrile

$N_4C_8H_{12}$

Ficel AZDN-FF
Nitrocel
Poly-Zole AZDN-FF
Porofor N
Vazo

Azodicarbonamide

$N_4O_2C_2H_4$

Azobisformamide,1,1'-
Azocel
Celogen AZ
Ficel AC
Kempore
Porofor ADC
Vinyfor AC

Black PN

$N_5O_{14}S_4C_{28}H_{21}\cdot 4Na$

1743 Black CI
C.I. 28440 CI
Black PN CI
Blue Black BN CI
Brillant Black BN
Brilliant Acid Black BNA Export CI
Brilliant Acid Black BN Extra Pure A CI
Brilliant Black
Brilliant Black A CI
Brilliant Black BN CI
Brilliant Black N.FQ CI
Brilliant Black NAF
Certicol Black PNW CI
Cilefa Black B CI
C.I. Food Black 1, tetrasodium salt
Edicol Supra Black BN CI
Hexacol Black PN CI
Melan Black CI
1,7-Naphthalenedisulfonic acid, 4-acetamido-5-hydroxy-6-[[7-sulfo-4-[(p-sulfophenyl)azo]-1-naphthyl]azo]-, tetrasodium salt CI
Tetrasodium 2-[4-(p-sulfophenylazo)-7-sulfo-1-naphthylazo]-8-acetamido-1-naphthol-3,5-disulfonate
Xylene Black F

Brilliant Black

$N_4O_{13}S_4C_{30}H_{20}\cdot 4Na$

C.I. 27260 CI
Brilliant black
C.I. Acid Black 3 CI
C.I. Acid Black 3, tetrasodium salt CI
Naphthol Black 3B CI
Tertracid Brilliant Black B CI

Brown FK

C.I. Food Brown 1

HUE **Yellowish Brown**

Edicol Supra Brown OH ...
Golden Brown KBS ... ...
Hexacol Brown FK ... ...

Consists essentially of a mixture of the disodium salt of 4, 4′-(4, 6-diamino-*m*-phenylenebisazo) dibenzenesulfonic acid and the sodium salt of 4-(4, 6-diamino-*m*-tolylazo) benzenesulfonic acid

Butter Yellow

$N_3C_{14}H_{15}$

C.I. 11020 CI
Aniline, N,N-dimethyl-p-(phenylazo)-
Azobenzene, p-dimethylamino-
Benzeneazodimethylaniline
Brilliant Fast Oil Yellow CI
Brilliant Fast Spirit Yellow CI
Brilliant Oil Yellow CI
Butter or methyl yellow MERCK
Butter yellow
Cerasine Yellow GG CI
C.I. Solvent Yellow 2
DAB
Dimethylaminoazobenzene CDF
Dimethylaminoazobenzene
N,N-Dimethyl-4-aminoazobenzene
p-Dimethylaminoazobenzene MERCK
4-Dimethylaminoazobenzene
4-(N,N-Dimethylamino)azobenzene
4-Dimethylaminoazobenzol
4-Dimethylaminophenylazobenzene
N,N-Dimethyl-p-phenylazoaniline
Dimethyl Yellow CI
DMAB
Enial Yellow 2G CI
Fast Oil Yellow B CI
Fat Yellow CI
Fat Yellow A CI
Fat Yellow R CI
Fat Yellow AD OO CI
Fat Yellow ES CI
Fat Yellow ES Extra CI
Fat Yellow extra conc CI
Grasal Brilliant Yellow CI
Methyl yellow
Oil Yellow CI
Oil Yellow D CI
Oil Yellow G CI
Oil Yellow 2G CI
Oil Yellow 20 CI
Oil Yellow 2625 CI
Oil Yellow BB CI
Oil Yellow FN CI
Oil Yellow GG CI
Oil Yellow GR CI
Oil Yellow II CI
Oil Yellow PEL CI
Oleal Yellow 2G CI
Organol Yellow ADM CI
Orient Oil Yellow GG CI
Petrol Yellow WT CI
Resinol Yellow GR CI
Silotras Yellow T2G CI
Somalia Yellow A CI
Stear Yellow JB CI
Sudan Yellow GG CI
Sudan Yellow GGA CI
Toyo Oil Yellow G CI
Waxoline Yellow ADS
Yellow G Soluble in Grease CI

C.I. Solvent Yellow 2

HUE Yellow→Reddish Yellow

Brilliant Fast Oil Yellow ...
Brilliant Fast Spirit Yellow ...
Brilliant Oil Yellow ... ...
Cerasine Yellow GG ... ...
Dimethyl Yellow ... ...

Butter Yellow cont.

Fast Oil Yellow B ...
Fat Yellow ... ...
Fat Yellow extra conc. ...
Fat Yellow A ... ...
Fat Yellow AD OO ...
Fat Yellow ES extra ...
Fat Yellow R ... ...
Fat Yellow R (8186) ...
Grasal Brilliant Yellow...
Oil Yellow ... ...

Oil Yellow 20 ... ...
Oil Yellow 2625 ... ...
Oil Yellow 7463 ... ...
Oil Yellow II ... ...
Oil Yellow BB ... ...
Oil Yellow D ... ...
Oil Yellow DN ... ...
Oil Yellow FN ... ...
Oil Yellow G ... ...
Oil Yellow 2G ... ...
Oil Yellow GG ... ...
Oil Yellow GR ... ..
Oil Yellow N ... ...
Organol Yellow ADM ...
Petrol Yellow WT ...
Somalia Yellow A ...
Stéar Yellow JB ... ...
Sudan Yellow GG ...
Sudan Yellow GG ...
Sudan Yellow GGA ...
Waxoline Yellow AD ...
Yellow G Soluble in Grease

Chrysoidine Base

$N_4C_{12}H_{12}$

Chrysoidine
Chrysoidine Base CI
Chrysoidine Base A CI
Chrysoidine Base B CI
Chrysoidine G Base CI
Chrysoidine J Base CI
Chrysoidine Y Base CI
Chrysoidine Y Base New CI
Chrysoidine YD Base CI
C.I. Basic Orange 2
C.I. Basic Orange 2, free base CI
C.I. Solvent Orange 3 CI
C.I. Solvent Orange 34 CI
2,4-Diaminoazobenzene
Fat Brown GG CI
Grasan Chrysoidine CI
Waxoline Orange Y CI

Chrysoidine R

$N_4C_{12}H_{12}\cdot HCl$

C.I. Basic Orange 1

HUE Dull Yellowish Orange
ARTIFICIAL LIGHT: brighter

Brasilazina Orange 3R ... ...
Calcozine Orange RS ... ...
Chrysoidine R .. ..
Chrysoidine R (*Biological stain and indicator*) . ...
Chrysoidine RN ... .
Chrysoidine 3R .. . ...
Chrysoidine 3RN .. ..
Chrysoidine RPL . . ..
Chrysoidine RRS ... ...
Chrysoidine RS ... ...
Chrysoidine RS ... ...
Diazocard Chrysoidine R ... ...
Pure Chrysoidine RD ... ...
Tertrophene Brown CR... ... ...

Chrysoidine Y Special

$N_5O_6SC_{29}H_{19}\cdot Na_2$

C.I. Basic Orange 2

HUE Yellowish Orange→Orange
ARTIFICIAL LIGHT: Brighter

Brasilazina Orange Y ... ...
Calcozine Orange YS ... ...
Chrysoidine ... ...
Chrysoidine ... .
Chrysoidine A ... ...
Chrysoidine B ... ...
Chrysoidine G ... ...
Chrysoidine GN ... ...

Chrysoidine GS ... ...
Chrysoidine HR ... ...
Chrysoidine J ... ...
Chrysoidine J ... ...
Chrysoidine M, PRL & PRR...
Chrysoidine SL ... ...
Chrysoidine SS ... .
Chrysoidine Y ... ...
Chrysoidine Y Base New ... ...
Chrysoidine Y Special (*Biological stain and indicator*) ... ...
Chrysoidine YL .. ..
Diazocard Chrysoidine G . . . .
Leather Orange HR ... ...
Nippon Kagaku Chrysoidine ... ...
Pure Chrysoidine YD ... ...
Sugai Chrysoidine ... .
Tertrophene Brown CG .. ..

Coccine

$N_2O_{10}S_3C_{20}H_{14}.3Na$

1578 Red CI
C.I. 16255 CI
Acidal Bright Ponceau 3R CI
Acid Brilliant Scarlet 3R CI
Acid Ponceau 4R CI
Acid Red 18 CI
Acid Scarlet 3R CI
Acid Scarlet 3RZ CI
Acid Scarlet 4R CI
Acilan Scarlet V3R CI
Aizen Brilliant Scarlet 3RH CI
Atul Acid Scarlet 3R CI
Atul Scarlet F CI
Brilliant Ponceau 3R CI
Brilliant Ponceau 3RF CI
Brilliant Ponceau 4R CI
Brilliant Ponceau 4RC CI
Brilliant Ponceau 5R CI
Brilliant Ponceau 4RC Specially Pure CI
Brilliant Scarlet
Brilliant Scarlet 3R CI
Brilliant Scarlet 4R CI
Brilliant Scarlet 3R (Biological stain) CI
Brilliant Scarlet 3R Conc
Bucacid Brilliant Scarlet 3R CI
Calcocid Brilliant Scarlet 3RN CI
Certicol Ponceau 4RS CI
Cilefa Ponceau 4R CI
Coccine
Coccin Red CI
Cochineal Red A CI
Cochineal Red 4R CI
Cochineal Red A Specially Pure CI
Colacid Ponceau 4R CI
C.I. Acid Red 18 CI,MERCK
C.I. Acid Red 18, trisodium salt
C.I. Food Red 7 CI
Curol Bright Red 4R CI
Daishiki Brilliant Scarlet 3R CI
Edicol Supra Ponceau 4R CI
Eurocert Cochineal Red A CI
Fenazo Scarlet 3R CI
Food Red 6 CI
Food Red 7 CI
HD Ponceau 4R CI
HD Ponceau 4R Supra CI
Hexacol Ponceau 4R CI
Hidacid Fast Scarlet 3R CI
Hispacid Brilliant Scarlet 3RF CI
Java Scarlet 3R CI
Kayaku Acid Brilliant Scarlet 3R CI
Kayaku Food Colour Red No. 102 CI
Kiton Scarlet 4R CI
Kochineal Red A for Food CI
1,3-Naphthalenedisulfonic acid, 7-hydroxy-8-[(4-sulfo-1-naphthyl)azo]-, trisodium salt
Naphthalene Ink Scarlet 4R CI
Naphthalene Scarlet 4R CI
Naphthalene Scarlet 4RS
Neklacid Red 3R CI
Neklacid Red 4R CI
New Coccin CI
New Coccine CI
New Coccine Extra Conc. A Export CI
New Coccine Extra Pure A CI
Ponceau 3R CI
Ponceau 4R CI
Ponceau 4RE
Ponceau 4RF CI
Ponceau 4RT CI

Coccine cont.

Ponceau 4R Aluminum Lake CI
Ponceau 4RE.FQ CI
Pontacyl Scarlet RR CI
Purple Red CI
Rakuto Brilliant Scarlet 3R CI
San-ei Brilliant Scarlet 3R CI
Scarlet 4R CI
Scarlet 4RA CI
Strawberry Red A Geigy CI
Sugai Brilliant Scarlet 3R CI
Symulon Acid Brilliant Scarlet 3R CI
Takaoka Brilliant Scarlet 3R CI
Trisodium 1-(4-sulfo-1-naphthylazo)-2-naphthol-6,8-disulfonate
Victoria Scarlet 3R
Victoria Scarlet Red

Congo Red

$N_6O_6S_2C_{32}H_{24}.2Na$
C.I. 22120 CI
Atlantic Congo Red CI
Atul Congo Red CI
Atocard Red Congo CI
Benzo Congo Red CI
Brasilamina Congo 4B CI
C.I. Direct Red 28 CI
C.I. Direct Red 28, disodium salt
Congo red CDF
Congo Red MERCK,CI
Congo Red H CI
Congo Red L CI
Congo Red M CI
Congo Red N CI
Congo Red R CI
Congo Red W CI
Congo Red 4B CI
Congo Red 4BX CI
Congo Red CR CI
Congo Red ICI CI
Congo Red RS CI
Cotton Red L CI
Cotton Red 4BC CI
Cotton Red 5B CI
Diacotton Congo Red CI
Direct Red C CI
Direct Red K CI
Direct Red 28 CI
Direct Red DC-CF CI
Erie Congo 4B CI
Hispamin Congo 4B CI
Kayaku Congo Red CI
Mitsui Congo Red CI
1-Naphthalenesulfonic acid, 3,3'-[4,4'-biphenylenebis(azo)]
bis[4-amino-, disodium salt
Peeramine Congo Red CI
Sodium diphenyldiazo-bis-α-naphthylaminesulfonate MERCK
Sugai Congo Red CI
Tertrodirect Red C CI
Trisulfon Congo Red CI
Vondacel Red CL CI

Evans Blue

$N_6O_{14}S_4C_{34}H_{24}\cdot 4Na$

T 1824 MERCK
C.I. 23860 CI
Azovan Blue
4,4'-Bis[7-(1-amino-8-hydroxy-2,4-disulfo)naphthylazo]-3,3'-bitolyl tetrasodium salt MERCK
C.I. Direct Blue 53 CI
C.I. Direct Blue 53, tetrasodium salt
Diazol Pure Blue BF CI
Dye evens blue CARF
Evans Blue USP,ADI,MERCK,USP-A
Evans Blue dye
1,3-Naphthalenedisulfonic acid, 6,6'-[(3,3'-dimethyl-4,4'-biphenylylene)bis(azo)]bis[4-amino-5-hydroxy-, tetrasodium salt

3'-Methyl-4-dimethylaminoazobenzene

$N_3C_{15}H_{17}$

Aniline, N,N-dimethyl-p-(m-tolylazo)-
Aniline, N,N-dimethyl-4-(m-tolylazo)-
3'M-DAB
3'Methyl-DAB
4-(N,N-Dimethylamino)-3'-methylazobenzene
4-Dimethylamino-3'-methylazobenzene CDF
N,N-Dimethyl-p-(m-tolylazo)aniline
MDAB CDF
3'-MDAB
3'-Methyl butter yellow
3'-Methyl-4-dimethylaminoazobenzene

Methyl Orange

$N_3O_3SC_{14}H_{15}\cdot Na$

C.I. 13025 CI
Benzenesulfonic acid, p-[[p-(dimethylamino)phenyl]azo]-, monosodium salt
C.I. Acid Orange 52, monosodium salt CI
Eniamethyl Orange CI
Gold orange MERCK
Helianthine B MERCK
KCA Methyl Orange CI
Methyl Orange CI
Methyl Orange B CI
Methyl orange sodium salt MERCK,CI
Orange III MERCK
Sodium p-dimethylaminoazobenzenesulfonate MERCK
Tropaeolin D MERCK

Methyl Red

$N_3O_2C_{15}H_{15}$

Benzoic acid, o-[[p-(dimethylamino)phenyl]azo]-
C.I. Acid Red 2
p-(Dimethylamino)azobenzene-o-carboxylic acid
Methyl red CI

Neoprontosil

$N_4O_{10}S_3C_{18}H_{14}\cdot 2Na$

Bayer 102
6-Acetamido-4-hydroxy-3-[(p-sulfamoylphenyl)azo]-2,7-naphthalenedisulfonic acid disodium salt
Azosulfamide IECMTN,ADI,MERCK
Disodium 2-(4'-sulfamylphenylazo)-7-acetamido-1-hydroxynaphthalene-3,6-disulfonate MERCK,IECMTN
Drometil MERCK
Leuconeoprontosil
2,7-Naphthalenedisulfonic acid, 6-acetamido-4-hydroxy-3-[(p-sulfamoylphenyl)azo]-, disodium salt
Neoprontosil ADI,MERCK
Neoprontosil sodium
Prontosil S MERCK
Prontosil Soluble MERCK
Streptocid Rubrim
Streptozon S
Streptozon II

Niagara Blue 2B

$N_6O_{14}S_4C_{32}H_{24}\cdot 4Na$

Blue 2B CI
C.I. 22610 CI
Airedale Blue 2BD CI
Aizen Direct Blue 2BH CI
Amanil Blue 2BX CI
Atlantic Blue 2B CI
Atul Direct Blue 2B CI
Azocard Blue 2B CI
Azomine Blue 2B CI
Belamine Blue 2B CI
Bencidal Blue 2B CI
Benzanil Blue 2B CI
Benzo Blue BBA-CF CI
Benzo Blue BBN-CF CI
Benzo Blue GS CI
Blue 2B salt
Brasilamina Blue 2B CI
Calcomine Blue 2B CI
Chloramine Blue 2B CI
Chlorazol Blue B CI
Chlorazol Blue BP CI
Chrome Leather Blue 2B CI
C.I. Direct Blue 6 CI
C.I. Direct Blue 6, tetrasodium salt
Cresotine Blue 2B CI
Diacotton Blue BB CI
Diamine Blue 2B
Diamine Blue BB CI
Diaphtamine Blue BB CI
Diazine Blue 2B CI
Diazol Blue 2B CI
Diphenyl Blue M2B CI
Diphenyl Blue 2B CI
Diphenyl Blue KF CI
Direct Blue A CI
Direct Blue K
Direct Blue M2B CI
Direct Blue 2B CI
Direct Blue GS CI
Enianil Blue 2BN CI
Fenamin Blue 2B CI
Fixanol Blue 2B CI
Hispamin Blue 2B CI
Indigo Blue 2B CI
Kayaku Direct Blue BB CI
Mitsui Direct Blue 2BN CI

Niagara Blue 2B cont.

Naphtamine Blue 2B CI
2,7-Naphthalenedisulfonic acid, 3,3'-[4,4'-biphenylylenebis (azo)]bis[5-amino-4-hydroxy-, tetrasodium salt
Niagara Blue 2B CI
Nippon Blue BB CI
Paramine Blue 2B CI
Phenamine Blue BB CI
Pheno Blue 2B CI
Pontamine Blue BB CI
Tertrodirect Blue 2B CI
Vondacel Blue 2B CI

Niagara Sky Blue 6B

$N_6O_{16}S_4C_{34}H_{28}\cdot 4Na$
C.I. 24410 CI,PI
Afredale Blue FFD CI
Amanil Sky Blue 6B CI
Amanil Sky Blue FF CI
Atlantic Resin Fast Blue LLGG CI
Atlantic Sky Blue 6B CI
Atlantic Sky Blue FF CI
Atul Direct Sky Blue FB CI
Azine Brilliant Blue 6B CI
Azocard Blue 6B CI
Belamine Sky Blue FF CI
Benzanil Sky Blue FF CI
Benzanil Supra Blue 2GN CI
Benzo Brilliant Blue 6BS CI
Brasilamina Sky Blue 6B CI
Brilliant Benzo Blue 6BA-CF CI
Calcodur Blue 6GFL CI
Calcodur Resin Fast Blue 6G CI
Calcomine Sky Blue FF CI
Chicago Blue 6B CI
Chicago Sky Blue 6B CI
Chloramine Sky Blue FF CI
Chlorantine Fast Blue B5GL CI
Chlorazol Sky Blue FF PI,CI
Chrome Leather Sky Blue GS CI
C.I. Direct Blue 1 CI
C.I. Direct Blue 1, tetrasodium salt
Cresotine Blue 6B CI
Diacotton Sky Blue 6B CI
Diaphtamine Blue BS CI
Diazine Sky Blue FF CI
Diazol Pure Blue 6B CI
Diphenyl Brilliant Blue FF CI
Direct Blue 6B CI
Direct Blue 6BS CI
Direct Blue FF CI
Direct Blue FFN CI
Direct Bright Blue
Direct Brilliant Blue FF CI
Direct Brilliant Blue MFF CI
Direct Brilliant Sky Blue 6B CI
Direct Pure Blue 6B CI
Direct Pure Blue FF CI
Direct Sky Blue 6B CI
Direct Sky Blue 6BS CI
Direct Sky Blue FF CI
Direct Sky Blue Green Shade CI
Direct Sky Blue GS CI
Enianil Brilliant Blue FF CI
Fastusol Brilliant Blue L8GU CI
Fenamin Sky Blue 3F CI
Fixanol Sky Blue FF CI
Hispamin Sky Blue 6B CI

Niagara Sky Blue 6B cont.

Ink Blue 6B CI
Japanol Brilliant Blue 6BKX CI
Kayaku Direct Sky Blue 6B CI
Lumicrease Blue 4GL CI
Lumicrease Sky Blue 6GUL CI
Mitsui Direct Brilliant Blue 6B CI
Naphtamine Sky Blue DD CI
Niagara Sky Blue 6B CI
Nyanza Sky Blue 6B CI
Paper Blue 6B CI
Paramine Sky Blue FF CI
Phenamine Brilliant Blue 6B CI
Pheno Sky Blue 6BX CI
Pontamine Sky Blue
Pontamine Sky Blue 6BX CI
Pontamine sky blue 6x
Pontamine Sky Blue 6BX Greenish CI
Pure Sky Blue 6B CI
Pyrazol Fast Brilliant Blue VP CI
Shikiso Direct Sky Blue 6B CI
Sirius Supra Blue 4G CI
Sky Blue 6B
Solar Blue 4GL CI
Tertrodirect Blue FF CI
Vegentine Blue CSW CI
Vondacel Blue FF CI

Orange 1

$N_2O_4SC_{16}H_{12}.Na$

C.I. 14600 CI
Acid Leather Orange I CI
Acid Orange I
Aizen Orange 1 CI
Benzenesulfonic acid, p-[(4-hydroxy-1-naphthyl)azo]-, sodium salt
Certiqual Orange I CI
C.I. Acid Orange 20
Dye orange No. 1 CARF
Enlacid Orange I CI
Ext. D and C Orange No. 3 CI
Hispacid Orange 1 CI
Java Orange I CI
Naphthalene Orange I CI
α-Naphthol orange MERCK
Neklacid Orange 1 CI
Orange I CI,MERCK
1333 Orange CI
Orange I Extra Conc. A Export CI
Orange IM CI
A.F. Orange No. 1 CI
Sodium azo-α-naphtholsulfanilate MERCK
4-p-Sulfophenylazo-1-naphthol monosodium salt CARF
Tertracid Orange I CI
Tropaeolin 000 no. 1 MERCK

Orange G

$N_2O_7S_2C_{16}H_{12}.2Na$

C.I. 16230 CI
Acidal Fast Orange CI
Acid Fast Orange G CI
Acid Fast Orange EGG CI
Acid Leather Orange KG CI
Acid Leather Orange PGW CI
Acid Orange G CI
Acid Orange 2G CI
Acid Orange 10 CI
Acilan Orange GX CI
Amacid Crystal Orange CI
Apocid Orange 2G CI

C.I. Acid Orange 10

Acidal Fast Orange ... ...
Acid Fast Orange G ... ...
Acid Light Orange J ... ...
Acid Light Orange SX ... ...
Acid Orange G ... ...
Acid Orange GG... ... ...
Acid Orange GG... ... ...
Acilan Orange GX ... ...
Apocid Orange 2G ... ...
Brasilan Orange 2G ... ...
Calcocid Fast Light Orange 2G
Cetil Light Orange GG ...
Crystal Orange 2G ... ...
Eniacid Light Orange G ...
Erio Fast Orange AS ... ...
Fast Acid Orange G ... ...
Fast Light Orange G ... ...

Fast Light Orange GA-CF ...
Fenazo Light Orange 2G ...
Hidacid Fast Orange G... ...
Hispacid Fast Orange 2G ...
Java Orange 2G ... ...
Kiton Fast Orange G ... ...
Kiton Fast Orange G ... ...
Leather Orange GG ... ...
Light Orange G ... ...
Light Orange G ... ...
Naphthalene Fast Orange 2G ...
Neklacid Fast Light Orange GG
Orange G ... ...
Du

Orange G (*Biological stain*) ...
Orange G (*Indicator*) ... ...
Orange G, BPC ... ...
Orange GG ... ...
Orange 2G ... ...
Solar Light Orange GX ...
Tertracid Light Orange G ...
Wool Orange 2G ... ...
Xylene Fast Orange G ... ...

C.I. Food Orange 4

Acid Light Orange JA Export .
Acid Orange G ... .
Acid Orange GG Crystals .
Dolkwal Orange G ... .
Hexacol Orange G ... .
Hexacol Orange GG Crystals .
Light Orange AG Conc. .
Orange G ... ...
Orange GG ... .
Orange GG Specially Pure .

Orange RN

$N_2O_4SC_{16}H_{12}.Na$

C.I. 15970 CI
Acidine Orange GN CI
Acilan Orange G CI
Acilan Ponceau 4GBL CI
Amacid Brilliant Orange CI
Brilliant Orange CI
Brilliant Orange G CI
Brilliant Orange GN CI
Brilliant Orange GN Type 8019 CI
C.I. Acid Orange 12 CI
C.I. Acid Orange 12, sodium salt CI
C.I. Food Orange 1 CI
Croceine Orange
Croceine Orange Y CI
Croceine Orange 2G CI
Croceine Orange EN CI
Crocein Orange CI
Helio Orange CAG CI
Hexacol Orange RN CI
Hispacid Orange CG CI
Kiton Brilliant Orange G CI
Kiton Ponceau 4G CI
Lutetia Orange 2JR CI
Monolite Orange C CI
2-Naphthalenesulfonic acid, 6-hydroxy-5-(phenylazo)-, sodium salt
Orange G CI
1008 Orange CI
Orange G Food Grade CI
Orange LZS CI
Orange RN CI
Ponceau 4G CI
Segnale Light Orange GR CI
Siloton Orange GR CI
Tertracid Brilliant Orange P4G CI

Orange SS

$N_2OC_{17}H_{14}$

C.I. 12100 CI
C.I. Solvent Orange 2 CI
Dolkwal Orange SS CI
Ext. D and C Orange No. 4 CI
Fat Orange II CI
Fat Orange RR CI
FD and C Orange No. 2 LC
Hexacol Oil Orange SS CI
Lacquer Orange V CI
2-Naphthol, 1-(o-tolylazo)-
Oil Orange OPEL CI
Oil Orange O'PEL
Oil Orange SS CI
Oil Orange TX CI
Oleal Orange SS CI
A.F. Orange No. 2 CI
Orange OT*
Orange 3R Soluble in Grease CI
Orange SS LC
Organol Orange 2R CI
Toluene-2-azonaphthol-2
1-o-Tolylazo-2-naphthol

Ponceau 2R

$N_2O_7S_2C_{18}H_{16}.2Na$

1695 Red CI
C.I. 16150 CI
Acidal Ponceau G CI
Acid Leather Red P2R CI
Acid Leather Red KPR CI
Acid Leather Scarlet IRW CI
Acid Ponceau R CI
Acid Ponceau 2RL CI
Acid Ponceau Special CI
Acid Red 26 CI
Acid Scarlet 2B CI
Acid Scarlet 2R CI
Acid Scarlet 2RL CI
Acid Scarlet 2R for Lakes CI
Acid Scarlet 2R for Lakes Bluish CI
Ahcocid Fast Scarlet R CI
Alzen Ponceau RH CI
Amacid Lake Scarlet 2R CI
Calcocid Scarlet 2R CI
Calcolake Scarlet 2R CI
Certicol Ponceau MXS CI
Colacid Ponceau Special CI
C.I. Acid Red 26 CI
C.I. Acid Red 26, disodium salt CI
C.I. Food Red 5 CI
Disodium (2,4-dimethylphenylazo)-2-hydroxynaphthalene-3,6-disulfonate
Disodium salt of 1-(2,4-xylylazo)-2-naphthol-3,6-disulfonic acid
Edicol Supra Ponceau R CI
Fenazo Scarlet 2R CI
Hexacol Ponceau 2R CI
Hexacol Ponceau MX CI
Hidacid Scarlet 2R CI
Kiton Ponceau R CI
Kiton Ponceau 2R CI
Kiton Scarlet 2RC CI
Lake Scarlet R CI
Lake Scarlet 2RBN CI
2,7-Naphthalenedisulfonic acid, 3-hydroxy-4-(2,4-xylylazo)-, disodium salt
Naphthalene Lake Scarlet R CI
Naphthalene Scarlet R CI
Naphthazine Scarlet 2R CI
Neklacid Red RR CI
New Ponceau 4R CI
Paper Red HRR CI
Pigment Ponceau R CI
Ponceau G CI
Ponceau R CI
Ponceau 2R CI,LC
Ponceau 2RL CI
Ponceau 2RX CI
Ponceau R (Biological stain) CI
Ponceau 2R (Biological stain) CI
Ponceau BNA CI
Ponceau 2R Extra A Export CI
Ponceau MX CI
Ponceau PXM CI
Ponceau Red
Ponceau Red R
Ponceau RR CI
Ponceau RR Type 8019 CI
Ponceau RS
Ponceau Xylidine (Biological stain) CI

Ponceau 2R cont.

D+C Red No. 5 CI
Scarlet R CI
Scarlet 2R CI
Scarlet 2RB CI
Scarlet 2RL Bluish CI
Scarlet RRA CI
Tertracid Ponceau 2R CI
Xylidine Ponceau CI
Xylidine red
1-Xylylazo-2-naphthol-3,6-disulfonic acid, disodium salt
1-(2,4-Xylylazo)-2-naphthol-3,6-disulfonic acid disodium salt

Ponceau 3R

$N_2O_7S_2C_{19}H_{18}.2Na$
C.I. 16155 CI
C.I. Food Red 6 CI
C.I. Food Red 6, disodium salt CI
Dolkwal Ponceau 3R CI
Ext. D and C Red No. 15
External D and C Red No. 15 CI
FD and C Red No. 1 CI,LC
Maple Ponceau 3R CI
2,7-Naphthalenedisulfonic acid, 3-hydroxy-4-[(2,4,5-trimethylphenyl)azo]-, disodium salt CI
Ponceau 3R MERCK,CI,LC
Ponceau 3RN CI
Ponceau 3R Lake CI
Sodium cumeneazo-β-naphthol disulfonate MERCK
Usacert Red No. 1 CI

Ponceau 6R

$N_2O_7S_2C_{20}H_{14}.2Na$
C.I. 16250 CI
Acidal Crystals Ponceau CI
Acid Leather Ponceau 6R CI
Acid Ponceau 6R CI
Acid Red 6A CI
Colacid Red 6A CI
C.I. Acid Red 44
Crystal Ponceau CI
Crystal Ponceau M6R CI
Crystal Ponceau 6R CI
Crystal Scarlet 6R CI
Crystal Tertracid Ponceau 6R CI
1,3-Naphthalenedisulfonic acid, 7-hydroxy-8-(1-naphthylazo)-, disodium salt
Ponceare 6R
Ponceau 6R CI
Ponceau Cristallise Extra A Export CI
Ponceau Crystals 6R CI
Ponceau 6R Crystals CI

Ponceau SX

$N_2O_7S_2C_{18}H_{14}.2Na$

1306 Red CI
12101 Red CI
C.I. 14700 CI
Certicol Ponceau SXS CI
C.I. Food Red 1 CI
C.I. Food Red 1, disodium salt
Dye FD and C Red No. 4 CARF
Edicol Supra Ponceau SX CI
FD and C Red No. 4 CFR,CI
Food Red 4 CI
Hexacol Ponceau SX CI
1-Naphthalenesulfonic acid, 4-hydroxy-3-[(6-sulfo-2,4-xylyl)azo]-
Ponceau SX
2-(6-Sulfo-2,4-xylylazo)-1-naphthol-4-sulfonic acid, disodium salt
Usacert Red No. 4 CI

Prontosil

$N_4O_2SC_{12}H_{13}$

Benzenesulfonamide, p-[(2,4-diaminophenyl)azo]-
Chrysoidine, 4'-sulfamoyl-
2,4-Diaminoazobenzene-4'-sulfonamide
p-[(2,4-Diaminophenyl)azo]benzenesulfonamide
Prontosil
Prontosil red
Red streptocide
Streptocide MERCK
Sulfachrysoidine INN,INN-A
Sulfamidochrysoidine
4-Sulfamyl-2,4-diaminoazobenzene
Sulphachrysoidine

Sunset Yellow

$N_2O_4SC_{12}H_{11}.Na$

C.I. 13010 CI
C.I. 13011 CI
Acid Yellow FWA CI
Benzenesulfonic acid, p-[(p-aminophenyl)azo]-, sodium salt
C.I. Food Yellow 6 CI
C.I. Food Yellow 6, monosodium salt CI
Hexacol Yellow RFS CI
New Yellow GMF CI
Sunset Yellow CI
11648 Yellow CI
Yellow RFS CI

Sunset Yellow FCF

$N_2O_7S_2C_{16}H_{12}.2Na$

C.I. 15985 CI
Acid Yellow TRA CI
Atul Sunset Yellow FCF CI
Canacert Sunset Yellow FCF CI
Certicol Sunset Yellow CFS CI
Cilefa Orange S CI
C.I. Food Yellow 3 CI
C.I. Food Yellow 3, disodium salt CI
Dolkwal Sunset Yellow CI
Dye FDC yellow lake 6 CARF
Dye FDC yellow No. 6 CARF
Dye Sunset Yellow CARF
Edicol Supra Yellow FC CI
Enlacid Sunset Yellow CI
Eurocert Orange FCF CI
FD and C Yellow 6 CI
FD and C yellow lake No. 6 CARF
FD and C Yellow No. 6 CI,CFR,LC
Food Yellow 6 CI
HD Sunset Yellow FCF CI
HD Sunset Yellow FCF Supra CI
Hexacol Sunset Yellow FCF CI
Hexacol Sunset Yellow FCF Supra CI
KCA Foodcol Sunset Yellow FCF CI
Maple Sunset Yellow FCF CI
2-Naphthalenesulfonic acid, 6-hydroxy-5-[(p-sulfophenyl)azo]-, disodium salt CI
Orange II R
Orange PAL CI
Orange RGL conc. Specially Pure CI
Orange Yellow S.FQ CI
Para Orange
1-p-Sulfophenylazo-2-naphthol-6-sulfonic acid, disodium salt CFR
Sun Orange A Geigy CI
Sunset Yellow CI
Sunset Yellow FCF LC,CI
Sun Yellow
Sun Yellow Extra Conc. A Export CI
Sun Yellow Extra Pure A CI
Sun Yellow FCF
Usacert Yellow No. 6 CI
1351 Yellow CI
1899 Yellow CI
A.F. Yellow No.5 CI
Yellow Orange S
Yellow Sun
Yellow SY for Food CI

Tartrazine

$N_4O_9S_2C_{16}H_{12}.3Na$

C.I. 19140 CI
Acid Leather Yellow T CI
Acid Yellow T CI
Acid Yellow 23 CI
Acilan Yellow GG CI
Airedale Yellow T CI
Aizen Tartrazine CI
Amacid Yellow T CI
D and C Yellow No. 5 CI
Atul Tartrazine CI
Bucacid Tartrazine CI
Calcocid Yellow MCG CI
Calcocid Yellow XX CI
Canacert Tartrazine CI
3-Carboxy-5-hydroxy-1-p-sulfophenyl-4-p-sulfophenylazopyrazole trisodium salt MERCK,CFR
Certicol Tartrazol Yellow S CI
Cilefa Yellow T CI
C.I. Acid Yellow 23 CI
C.I. Acid Yellow 23, trisodium salt CI
C.I. Food Yellow 4 CI
Curon Fast Yellow 5G CI

Tartrazine

Dolkwal Tartrazine CI
Dye FD and C Yellow No. 5 CARF
Edicol Supra Tartrazine N CI
Egg Yellow A CI
Erio Tartrazine CI
Eurocert Tartrazine CI
FD and C Yellow 5 CI
FD and C Yellow No. 5 CI,MERCK,LC,CFR
Fenazo Yellow T CI
Food Yellow 5 CI
HD Tartrazine CI
HD Tartrazine Supra CI
Hexacert Yellow No. 5 CI
Hexacol Tartrazine CI
Hidazid Tartrazine CI
Hispacid Fast Yellow T CI
Hydrazine yellow MERCK
Hydroxine Yellow L CI
Kako Tartrazine CI
Kayaku Food Colour Yellow No. 4 CI
Kayaku Tartrazine CI
KCA Foodcol Tartrazine PF CI
KCA Tartrazine PF CI
Kiton Yellow T CI
Lake Yellow CI
Lemon Yellow A CI
Lemon Yellow A Geigy CI
Maple Tartrazol Yellow CI
Mitsui Tartrazine CI
Naphtocard Yellow O CI
Neklacid Yellow T CI
Oxanal Yellow T CI
San-ei Tartrazine CI
Sugai Tartrazine CI
Tartar Yellow N CI
Tartar Yellow S CI
Tartar Yellow FS CI
Tartar Yellow PF CI
Tartran Yellow CI
Tartraphenine CI
Tartrazine CI,MERCK
Tartrazine B CI
Tartrazine B.P.C. CI
Tartrazine G CI
Tartrazine M CI
Tartrazine N CI
Tartrazine O CI
Tartrazine T CI
Tartrazine A Export CI
Tartrazine Extra Pure A CI
Tartrazine FQ CI
Tartrazine Lake CI
Tartrazine Lake Yellow N CI
Tartrazine MCGL CI
Tartrazine NS CI
Tartrazine O Specially Pure CI
Tartrazine XX CI
Tartrazine XX Specially Pure CI
Tartrazine XXX CI
Tartrazine Yellow CI
Tartrazol BPC CI
Tartrazol Yellow CI
Tartrine Yellow O CI
Trisodium 3-carboxy-5-hydroxy-1-p-sulfophenyl-4-p-sulfophenylazopyraz=
ole MERCK
Unitertracid Yellow TE CI
Usacert Yellow No. 5 CI
Vondacid Tartrazine CI
Wool Yellow CI
Xylene Fast Yellow GT CI
1310 Yellow CI
1409 Yellow CI
Yellow Lake 69 CI
A.F. Yellow No.4 CI
Yellow No. 5 FDC CARF

Trypan Blue

$N_6O_{14}S_4C_{34}H_{28}\cdot 4Na$

Blue 3B CI
C.I. 23850 CI
Amanil Sky Blue R CI
Bencidal Blue 3R CI
Benzamine blue MERCK
Benzanil Blue 3BN CI
Benzo blue MERCK
Benzo Blue 3BS CI
Blue EMB CI
Brasilamina Blue 3B CI
Chloramine Blue 3B CI
Chrome Leather Blue 3B CI
C.I. Direct Blue 14 CI
C.I. Direct Blue 14, tetrasodium salt CI
Congo blue MERCK
Cresotine Blue 3B CI
Diamineblue MERCK
Diamine Blue 3B
Dianil blue MERCK
Diaphtamine Blue TH CI
Diazine Blue 3B CI
Diazol Blue 3B CI
Diphenyl Blue 3B CI
Direct Blue M3B CI
Direct Blue 3B CI
Hispamin Blue 3BX CI
2,7-Naphthalenedisulfonic acid, 3,3'-[(3,3'-dimethyl-4,4'-biphenylylene)bis(azo)]bis[5-amino-4-hydroxy-, tetrasodium salt
Naphthylamine blue MERCK
Niagara Blue MERCK
Niagara Blue 3B CI
Paramine Blue 3B CI
Pontamine Blue 3BX CI
Sodium ditolyldisazobis-8-amino-1-naphthol-3,6-disulfonate MERCK
Trypan blue CDF
Trypan Blue MERCK,VBB
Trypan Blue BPC CI
Trypane blue

Trypan Red

$N_6O_{15}S_5C_{32}H_{24}\cdot 5Na$

C.I. 22850 CI
2,7-Naphthalenedisulfonic acid, 4,4'-[(3-sulfo-4,4'-biphenylylene)bis(azo)]bis[3-amino-, pentasodium salt
Trypan Red MERCK,CI

TABLE 4.--DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971

[Dyes for which separate statistics are given in table 1 are marked below with an asterisk (*); dyes not so marked do not appear in table 1 because the reported data are accepted in confidence and may not be published. Manufacturers' identification codes shown below are taken from table 3. An x signified that the manufacturer did not consent to his identification with the designated product]

Dye	Manufacturers' identification codes (according to list in table 3)
ACID DYES	
*Acid yellow dyes:	
Acid Yellow 1	ACY.
Acid Yellow 3	ACS, ACY.
*Acid Yellow 11	ATL, BDO, CMG, VPC.
Acid Yellow 14	TRC.
*Acid Yellow 17	ACS, ACY, ATL, BDO, CMG, DUP, HN, PDC, SDH, TRC, VPC.
Acid Yellow 19	ATL.
*Acid Yellow 23	AAP, ACS, ACY, GAF, MRX, PDC, SDH, TRC, VPC, WJ, YAW.
Acid Yellow 25	GAF.
Acid Yellow 29	GAF, TRC.
*Acid Yellow 34	ACS, ATL, PDC.
*Acid Yellow 36	ACS, DUP, GAF, TRC.
*Acid Yellow 38	ACS, ATL, GAF.
*Acid Yellow 40	ALT, ATL, DUP, TRC, VPC.
*Acid Yellow 42	AAP, ACY, GAF, VPC.
*Acid Yellow 44	AAP, GAF, VPC.
Acid Yellow 49	VPC.
*Acid Yellow 54	ACS, ACY, CMG, GAF, HN, TRC, VPC.
Acid Yellow 59	VPC.
Acid Yellow 63	AAP, ACS.
Acid Yellow 65	ALT, FAB, TRC.
Acid Yellow 73	ACS, DUP, SDH.
*Acid Yellow 76	ACS, GAF, TRC.
Acid Yellow 77	ACY.
Acid Yellow 79	VPC.
*Acid Yellow 99	ACS, CMG, GAF, TRC, VPC.
Acid Yellow 114	CMG, TRC.
Acid Yellow 121	GAF.
*Acid Yellow 124	ACS, ATL, DUP, HN.
Acid Yellow 127	TRC.
Acid Yellow 128	ALT, TRC.
Acid Yellow 129	CMG, TRC.
Acid Yellow 135	GAF.
*Acid Yellow 151	ACY, ATL, CMG, DUP, FAB, GAF, HN, TRC, VPC.
Acid Yellow 152	ACY.
*Acid Yellow 159	ACS, ALT, FAB, GAF, HN, TRC, VPC.
Acid Yellow 174	DUP, VPC.
Acid Yellow 175	DUP.
Other acid yellow dyes	ACY, ALT, BAS, CMG, GAF, HST, TRC, VPC.
*Acid orange dyes:	
Acid Orange 1	GAF, HN.
Acid Orange 2	ACS.
Acid Orange 4	ACY.
Acid Orange 6	ACS.
*Acid Orange 7	AAP, ACS, ACY, ATL, CPC, DUP, GAF, HN, PDC, TRC, VPC, YAW.
*Acid Orange 8	ACS, ACY, ATL, DUP, GAF, HN, TRC, VPC.
*Acid Orange 10	ACS, ACY, ATL, DUP, GAF, PDC, TRC, VPC.
Acid Orange 12	ACS.
*Acid Orange 24	ACS, ACY, DUP, GAF, TRC, YAW.
Acid Orange 31	AAP.
Acid Orange 45	ACS, TRC.
Acid Orange 51	CMG, TRC.
Acid Orange 52	ACS, ATL.
Acid Orange 56	GAF.
*Acid Orange 60	ATL, CMG, DUP, GAF, HN, TRC.
Acid Orange 62	TRC.
Acid Orange 63	GAF, TRC.
*Acid Orange 64	ACS, ACY, DUP.
Acid Orange 69	ACY.

TABLE 4.--DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971--CONTINUED

Dye	Manufacturers' identification codes (according to list in table 3)
ACID DYES--Continued	
*Acid orange dyes--Continued	
Acid Orange 72	GAF.
*Acid Orange 74	ACS, CMG, GAF, TRC.
Acid Orange 76	TRC.
Acid Orange 85	ACS.
Acid Orange 86	ACS, ALT, TRC.
*Acid Orange 116	ACS, ALT, FAB, GAF, HN, TRC, YAW.
Acid Orange 119	TRC.
Acid Orange 128	DUP.
Acid Orange 132	DUP.
Other acid orange dyes	ALT, GAF, HST, TRC, VPC.
*Acid red dyes:	
*Acid Red 1	AAP, ACS, ACY, ATL, BDO, DUP, GAF, HN, SDH, TRC, VPC, YAW.
*Acid Red 4	AAP, ATL, BDO, CMG, GAF, PDC, TRC, VPC, YAW.
*Acid Red 14	ACS, ATL, GAF, PDC, YAW.
Acid Red 17	ACS, ATL, TRC.
*Acid Red 18	ACS, ATL, BDO, GAF, TRC.
*Acid Red 26	ACS, ACY, ATL, CPC.
Acid Red 27	ACS.
Acid Red 32	GAF.
Acid Red 33	YAW.
Acid Red 35	AAP, GAF.
*Acid Red 37	ATL, CMG, DUP, GAF, HN, TRC.
Acid Red 42	GAF.
Acid Red 52	GAF.
Acid Red 57	ATL, TRC.
Acid Red 66	AAP, ATL.
*Acid Red 73	ACS, ACY, ATL, DUP, GAF, PSC, TRC, YAW.
Acid Red 80	ATL, GAF, ICI.
*Acid Red 85	ACS, ACY, ALT. ATL, CMG, DUP, GAF, HN, TRC, VPC, YAW.
Acid Red 87	SDH.
*Acid Red 88	ACS, ACY, ATL, DUP, GAF, TRC, SDH, YAW.
*Acid Red 89	AAP, ATL, BDO, GAF, HN.
Acid Red 97	ATL, GAF.
*Acid Red 99	ATL, CMG, FAB, HN, TRC, YAW.
Acid Red 100	VPC.
Acid Red 106	YAW.
Acid Red 111	ATL.
*Acid Red 114	AAP, ACS, ALT, ATL, DUP, GAF, TRC, VPC.
*Acid Red 115	ACS, ATL, GAF.
*Acid Red 119	ALT, ATL.
Acid Red 133	GAF.
Acid Red 134	TRC.
*Acid Red 137	ACS, ATL, DUP, GAF, HN, TRC.
Acid Red 138	ALT.
*Acid Red 151	ACY, ALT, ATL, DUP, HN, TRC, VPC, YAW.
Acid Red 167	ACS, ATL, DUP, TRC.
Acid Red 175	DUP.
Acid Red 178	DUP.
*Acid Red 182	ACS, ALT, ATL, BDO, CMG, DUP, GAF, HN.
Acid Red 183	CMG, TRC.
*Acid Red 186	ACY, ATL, CMG, GAF, VPC.
Acid Red 191	TRC.
Acid Red 194	CMG, TRC.
Acid Red 201	TRC.
Acid Red 211	DUP.
Acid Red 212	TRC.
Acid Red 213	TRC.
*Acid Red 266	DUP, TRC, VPC.
Acid Red 292	ACY.
Acid Red 299	ALT, TRC.
Acid Red 309	TRC.

TABLE 4.--DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971--CONTINUED

Dye	Manufacturers' identification codes (according to list in table 3)
ACID DYES--Continued	
*Acid red dyes--Continued	
Acid Red 357	DUP, TRC, VPC.
Acid Red 345	DUP.
Acid Red 350	GAF.
Other acid red dyes	ALT, CMG, DUP, GAF, HN, TRC, VPC.
*Acid violet dyes:	
*Acid Violet 1	BDO, CMG, GAF.
*Acid Violet 3	ACS, ACY, TRC, YAW.
*Acid Violet 7	AAP, ACS, ATL, BDO, CMG, GAF, TRC, VPC.
*Acid Violet 12	BDO, CMG, DUP, GAF.
*Acid Violet 17	DUP, GAF, SDH.
Acid Violet 29	HSH.
Acid Violet 34	ATL, DUP, ICI.
Acid Violet 41	CMG.
*Acid Violet 43	ATL, HSH, ICI.
*Acid Violet 49	ACS, ACY, SDH, TRC.
Acid Violet 56	CMG, GAF.
Acid Violet 58	GAF.
Acid Violet 76	ACS.
Other acid violet dyes	TRC.
*Acid blue dyes:	
Acid Blue 1	ACS, GAF.
*Acid Blue 7	ACS, ACY, ATL, GAF, SDH.
*Acid Blue 9	ACS, GAF, SDH.
Acid Blue 10	AAP, ACS.
Acid Blue 15	GAF.
Acid Blue 20	ACS.
Acid Blue 23	TRC.
*Acid Blue 25	ACS, ATL, BDO, CMG, DUP, GAF, HN, TRC, VPC.
*Acid Blue 27	ALT, ATL, BDO, CMG, GAF.
Acid Blue 29	PDC, YAW.
Acid Blue 34	ACS.
*Acid Blue 40	ACS, ALT, ATL, BDO, DUP, GAF, ICI, TRC, VPC.
*Acid Blue 41	ACS, ALT, BDO, CMG, GAF.
Acid Blue 43	ACY, VPC.
*Acid Blue 45	ACS, ACY, ATL, CMG, DUP, GAF, HN, TRC.
Acid Blue 47	ICI.
Acid Blue 48	HSC.
*Acid Blue 62	ACS, ALT, BDO, CMG, GAF, VPC.
Acid Blue 69	GAF.
Acid Blue 74	ACS, DUP.
*Acid Blue 78	ALT, BDO, DUP, GAF, ICI, TRC.
*Acid Blue 80	ACS, ATL, TPC.
Acid Blue 81	ICI.
Acid Blue 83	GAF.
Acid Blue 89	ACS.
Acid Blue 90	TRC.
*Acid Blue 92	ACS, ATL, YAW.
Acid Blue 93	ACY, HSC.
Acid Blue 102	TRC.
Acid Blue 104	ACS, GAF.
*Acid Blue 113	ACS, ALT, ATL, BDO, CMG, DUP, FAB, GAF, HN, PDC
*Acid Blue 118	ACS, ATL, HN.
*Acid Blue 120	ACS, ATL, GAF, HN.
Acid Blue 122	DUP.
Acid Blue 129	CMG.
Acid Blue 145	ACS, DUP.
*Acid Blue 158 and 158A	BDO, GAF, HN, TRC, VPC.
Acid Blue 161	VPC.
Acid Blue 165	DUP.
Acid Blue 179	GAF.
Acid Blue 198	VPC.
Acid Blue 221	VPC.
*Acid Blue 230	ACS, DUP, TRC.
Acid Blue 231	TPC.

TABLE 4.--DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971--CONTINUED

Dye	Manufacturers' identification codes (according to list in table 3)
ACID DYES--Continued	
*Acid blue dyes--Continued	
Acid Blue 252	VPC.
Acid Blue 264	VPC.
Other acid blue dyes	ACY, ALT, ATL, CMG, GAF, HN, HST, TRC, VPC.
*Acid green dyes:	
Acid Green 1	ACS, ACY, DUP.
*Acid Green 3	ACS, ACY, GAF, TRC.
Acid Green 5	GAF.
*Acid Green 9	ACS, ACY, GAF.
Acid Green 12	ACS, GAF.
*Acid Green 16	ACS, GAF, TRC.
Acid Green 19	ALT.
*Acid Green 20	ACS, ATL, BDO, GAF, PDC, TRC.
Acid Green 22	GAF.
*Acid Green 25	ACS, ALT, ATL, CMG, GAF, HSH, ICI, TRC, VPC.
Acid Green 35	TRC.
Acid Green 41	ICI, VPC.
Acid Green 50	ACY, GAF.
Acid Green 58	TRC.
Acid Green 84	VPC.
Other acid green dyes	ALT, VPC.
*Acid brown dyes:	
Acid Brown 7	GAF.
Acid Brown 6	GAF.
*Acid Brown 14	AAP, ACY, DUP, GAF, TRC, YAW.
Acid Brown 19	TRC.
Acid Brown 22	DUP.
Acid Brown 28	TRC.
Acid Brown 31	GAF.
Acid Brown 45	TRC.
Acid Brown 96	ACY, CMG.
Acid Brown 97	ACY.
Acid Brown 98	ACY, TRC, YAW.
Acid Brown 152	GAF.
Acid Brown 158	GAF.
Acid Brown 213	GAF.
Other acid brown dyes	ACY, ALT, DUP, GAF, VPC.
*Acid black dyes:	
*Acid Black 1	AAP, ACS, ACY, ATL, DUP, GAF, HN, PDC, TRC, YAW.
Acid Black 2	ACS, ACY.
*Acid Black 24	ACS, CMG, DUP, GAF.
Acid Black 26, 26A and 26B	ATL, DUP, TRC.
Acid Black 29	GAF.
*Acid Black 48	ACY, ICI, TRC.
*Acid Black 52	ACS, ATL, DUP, GAF, HN, TRC.
Acid Black 58	CMG, DUP, TRC.
*Acid Black 60	BDO, TRC.
Acid Black 92	ACY.
*Acid Black 107	ACS, ALT, GAF, TRC.
Acid Black 108	GAF.
Acid Black 138	VPC.
Acid Black 140	CMG.
Other acid black dyes	ALT, ATL, HN, PDC, VPC, YAW.
AZOIC DYES AND COMPONENTS	
Azoic Compositions	
Azoic yellow dyes:	
Azoic Yellow 1	ATL, VPC.
*Azoic Yellow 2	ATL, VPC, BUC, x.
Azoic Yellow 3	PDC
Other azoic yellow dyes	ATL

TABLE 4.--DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971--CONTINUED

Dye	Manufacturers' identification codes (according to list in table 3)
AZOIC DYES AND COMPONENTS--Continued	
Azoic Compositions--Continued	
Azoic orange dyes:	
*Azoic Orange 3	ALL, ATL, BUC, x.
Azoic Orange 10	BUC.
Other azoic orange dyes	ATL.
Azoic red dyes:	
*Azoic Red 1	ALL, ATL, BUC, x.
*Azoic Red 2	ALL, ATL, BUC, GAF, x.
Azoic Red 6	ATL, BUC, x.
Azoic Red 16	ATL.
Azoic Red 73	GAF.
Azoic Red 74	GAF.
Other azoic red dyes	ALL, ATL, x.
Azoic violet dyes:	
Azoic Violet 1	ATL, BUC, GAF.
Other azoic violet dyes	ALL.
Azoic blue dyes:	
Azoic Blue 2	ATL.
*Azoic Blue 3	ALL, ATL. BUC, GAF, HST, x.
Azoic Blue 6	ATL.
Azoic Blue 7	GAF.
Azoic Blue 8	ALL.
Other azoic blue dyes	ALL, ATL.
Azoic green dyes:	
Azoic Green 1	ATL.
Other azoic green dyes	ALL, BUC, VPC.
Azoic brown dyes:	
Azoic Brown 3	x.
Azoic Brown 7	ATL, BUC.
*Azoic Brown 9	ALL, ATL, BUC, GAF, HST, VPC, x.
Azoic Brown 10	BUC.
Azoic Brown 26	GAF.
Other azoic brown dyes	ALL, ATL, GAF, VPC.
*Azoic black dyes:	
Azoic Black 1	HST.
Azoic Black 4	ATL, BUC, GAF.
Azoic Black 15	GAF.
Other azoic black dyes	ALL, ATL, GAF, VPC.
Azoic Diazo Components, Bases (Fast Color Bases)	
Azoic Diazo Component 2, base	ATL, BUC.
Azoic Diazo Component 3, base	BUC.
*Azoic Diazo Component 4, base	ALL, BUC, GAF, SDH.
Azoic Diazo Component 5, base	GAF, SDH.
Azoic Diazo Component 8, base	SDH.
Azoic Diazo Component 10, base	BUC, GAF.
Azoic Diazo Component 12, base	BUC, SDH.
Azoic Diazo Component 13, base	BUC.
Azoic Diazo Component 14, base	AAP.
Azoic Diazo Component 20, base	ALL, GAF.
Azoic Diazo Component 28, base	ALL, BUC, GAF.
*Azoic Diazo Component 32, base	AAP, ALL, ATL, BUC, SDH.
Azoic Diazo Component 34, base	SDH.
Azoic Diazo Component 44, base	BUC.
Azoic Diazo Component 46, base	ATL.
Azoic Diazo Component 48, base	CWN, GAF.

TABLE 4.--DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971--CONTINUED

Dye	Manufacturers' identification codes (according to list in table 3)
AZOIC DYES AND COMPONENTS--Continued	
Azoic Diazo Component 1, salt	
*Azoic Diazo Component 1, salt	AAP, ALL, BUC, GAF, SDH.
Azoic Diazo Component 2, salt	ALL, BUC.
*Azoic Diazo Component 3, salt	AAP, ALL, BUC, GAF, SDH.
Azoic Diazo Component 4, salt	ALL.
*Azoic Diazo Component 5, salt	AAP, ALL, BUC, GAF, SDH.
*Azoic Diazo Component 6, salt	AAP, BUC, GAF.
*Azoic Diazo Component 8, salt	AAP, ALL, BUC, GAF.
*Azoic Diazo Component 9, salt	AAP, ALL, BUC, GAF, SDH.
*Azoic Diazo Component 10, salt	ALL, BUC, GAF.
*Azoic Diazo Component 11, salt	AAP, ALL, BUC.
*Azoic Diazo Component 12, salt	AAP, ALL, BUC, GAF, SDH.
*Azoic Diazo Component 13, salt	AAP, ALL, BUC, GAF, SDH.
Azoic Diazo Component 14, salt	AAP.
Azoic Diazo Component 20, salt	ALL, BUC.
Azoic Diazo Component 28, salt	ALL, BUC, GAF, SDH.
Azoic Diazo Component 32, salt	ALL, SDH.
Azoic Diazo Component 34, salt	ALL, GAF.
Azoic Diazo Component 35, salt	BUC, GAF.
Azoic Diazo Component 36, salt	AAP, GAF.
Azoic Diazo Component 37, salt	GAF.
Azoic Diazo Component 41, salt	ALL, BUC.
Azoic Diazo Component 42, salt	GAF.
Azoic Diazo Component 44, salt	ALL, BUC.
Azoic Diazo Component 48, salt	BUC, SDH.
*Azoic Diazo Component 49, salt	AAP, ALL, BUC, GAF.
Azoic Diazo Component 121, salt	GAF.
Other azoic diazo components, salts	SDH.
Azoic Coupling Components (Naphthol AS and Derivatives)	
Azoic Coupling Component 2	ATL, BUC, GAF.
Azoic Coupling Component 3	BUC.
Azoic Coupling Component 4	ATL, BUC, GAF.
Azoic Coupling Component 5	BUC.
*Azoic Coupling Component 7	ALL, BUC, HST, SDH.
Azoic Coupling Component 8	ATL, BUC.
Azoic Coupling Component 10	ATL.
Azoic Coupling Component 11	ATL, BUC.
Azoic Coupling Component 12	BUC.
Azoic Coupling Component 13	GAF.
Azoic Coupling Component 14	ATL, BUC.
Azoic Coupling Component 15	ALL, BUC, GAF.
Azoic Coupling Component 16	BUC, GAF.
Azoic Coupling Component 17	ATL, BUC.
*Azoic Coupling Component 18	ALL, ATL, BUC, GAF.
Azoic Coupling Component 19	BUC, GAF.
Azoic Coupling Component 20	ATL, BUC, GAF.
Azoic Coupling Component 21	ATL, BUC.
Azoic Coupling Component 29	ATL, BUC.
Azoic Coupling Component 34	ATL, BUC.
Azoic Coupling Component 35	ALL, BUC.
*Azoic Coupling Component 43	ATL, BUC, GAF.
Azoic Coupling Component 44	PCW.
Azoic Coupling Component 107	HST.
Other azoic coupling components	ATL, GAF.

TABLE 4.--DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971--CONTINUED

Dye	Manufacturers' identification codes (according to list in table 3)
BASIC DYES	
*Basic yellow dyes:	
Basic Yellow 1	DUP.
Basic Yellow 2	ACS, ACY.
*Basic Yellow 11	ACS, ACY, ATL, DUP, FAB, GAF, TRC, VPC.
*Basic Yellow 13	ACS, ATL, DUP, GAF, VPC.
Basic Yellow 15	DUP.
Basic Yellow 21	VPC.
Basic Yellow 24	BAS.
Basic Yellow 25	BAS.
Basic Yellow 26	ACY.
Basic Yellow 28	VPC.
Basic Yellow 29	DUP, VPC.
Basic Yellow 31	DUP.
Basic Yellow 37	ACY.
Basic Yellow 41	ACY.
Basic Yellow 53	DUP.
Other basic yellow dyes	ATL, DUP, EKT, GAF, VPC.
*Basic orange dyes:	
*Basic Orange 1	ACS, ACY, DUP, GAF, PSC, TRC.
*Basic Orange 2	ACS, ACY, DSC, DUP, GAF, PSC, TRC.
Basic Orange 14	GAF.
*Basic Orange 21	ACS, ACY, ALT, ATL, DUP, FAB, GAF, TRC, VPC.
Basic Orange 22	ACS, GAF.
Basic Orange 24	DUP.
Basic Orange 25	DUP.
Basic Orange 26	DUP.
Basic Orange 27	VPC.
Basic Orange 28	VPC.
Basic Orange 31	ACY.
Basic Orange 39	DUP.
Other basic orange dyes	ATL.
*Basic red dyes:	
Basic Red 1	BAS, DUP.
Basic Red 2	ACS, DUP.
*Basic Red 9	ACY, DSC, HSC.
Basic Red 12	DUP.
*Basic Red 13	ACS, ATL, GAF, TRC, VPC.
*Basic Red 14	ACS, ACY, ATL, DUP, GAF, VPC.
Basic Red 15	ATL, DUP, GAF, TRC.
Basic Red 16	DUP.
Basic Red 17	DUP.
Basic Red 18	ATL, DUP, GAF, VPC.
Basic Red 19	DUP.
Basic Red 22	ACY, TRC.
Basic Red 23	VPC.
Basic Red 29	BAS.
Basic Red 30	ACY.
Basic Red 48	DUP.
Basic Red 49	DUP, GAF.
Other basic red dyes	ATL, DUP, EKT, VPC.
*Basic violet dyes	
*Basic Violet 1	ACS, ACY, DSC, DUP, HSC.
Basic Violet 2	DSC.
Basic Violet 3	ACS, DSC, DUP, SDH.
Basic Violet 4	DSC, DUP.
Basic Violet 7	ATL, GAF.
*Basic Violet 10	ACY, DUP, GAF.
Basic Violet 13	DSC.
Basic Violet 14	ACY, DSC.
Basic Violet 15	DUP.
*Basic Violet 16	ATL, DUP, FAB, GAF, TRC, VPC.
Basic Violet 18	ACY.
Basic Violet 24	DUP.
Basic Violet 27	ATL.

TABLE 4.--DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971--CONTINUED

Dye	Manufacturers' identification codes (according to list in table 3)
BASIC DYES--Continued	
*Basic blue dyes:	
*Basic Blue 1	DSC, GAF, SDH, VPC.
Basic Blue 2	DSC.
Basic Blue 3	ACY, DUP, GAF, HST.
Basic Blue 4	DUP.
*Basic Blue 5	DSC, SDH, VPC.
Basic Blue 6	ACY.
*Basic Blue 7	DSC, DUP, SDH.
Basic Blue 9	ACS, ACY, DUP.
Basic Blue 11	DSC, DUP, SDH.
Basic Blue 21	DUP.
Basic Blue 22	ACS, DUP, VPC.
Basic Blue 26	DSC, DUP, SDH.
Basic Blue 35	DUP.
Basic Blue 41	TRC.
Basic Blue 45	VPC.
Basic Blue 47	VPC.
Basic Blue 54	ACY, BAS.
Basic Blue 60	GAF.
Basic Blue 69	VPC.
Basic Blue 75	EKT.
Basic Blue 76	ACY.
Basic Blue 77	DUP.
Basic Blue 82	DUP, TRC.
Basic Blue 87	DUP.
Basic Blue 97	DUP.
Other basic blue dyes	ALT, BAS, DUP, EKT, VPC.
Basic green dyes:	
*Basic Green 1	ACS, ACY, DSC, DUP.
Basic Green 3	DUP.
*Basic Green 4	ACS, ACY, DSC, SDH, VPC.
Basic Green 7	DSC.
Other basic green dyes	VPC.
Basic brown dyes:	
*Basic Brown 1	ACS, ACY, DUP, GAF, PSC, TRC.
Basic Brown 2	GAF.
*Basic Brown 4	ACS, ACY, DSC, DUP, GAF, PSC, TRC.
Basic black dyes:	
Basic Black 9	VPC.
Other basic black dyes	ALT, DSC.
DIRECT DYES	
*Direct yellow dyes:	
*Direct Yellow 4	ACS, ACY, ATL, DUP, GAF, HN, TRC, VPC.
*Direct Yellow 5	ACS, ACY, GAF.
*Direct Yellow 6	ACS, ACY, DUP, GAF, TRC.
Direct Yellow 7	ATL.
Direct Yellow 8	ACS, ATL, GAF.
Direct Yellow 9	ATL.
*Direct Yellow 11	ACS, ACY, ALT, DUP, GAF, HN, TRC, VPC.
*Direct Yellow 12	ACS, ATL, DUP, FAB, GAF, TRC.
Direct Yellow 20	TRC.
Direct Yellow 23	DUP.
Direct Yellow 26	ALT, ATL, HN.
Direct Yellow 27	GAF.
*Direct Yellow 28	ACS, ATL, DUP, GAF, PDC, TRC.
*Direct Yellow 29	ATL, DUP, GAF.
Direct Yellow 34	ALT, HN.
Direct Yellow 39	TRC.
Direct Yellow 41	ATL.
*Direct Yellow 44	ACS, ALT, ATL, DUP, FAB, GAF, HN, TRC, VPC.
*Direct Yellow 50	ALT, ATL, DUP, FAB, GAF, HN, HSH, TRC, VPC.
Direct Yellow 59	DUP.

TABLE 4.--DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971--CONTINUED

Dye	Manufacturers' identification codes (according to list in table 3)
DIRECT DYES--Continued	
*Direct yellow dyes--Continued	
Direct Yellow 65	DUP.
Direct Yellow 81	ATL.
*Direct Yellow 84	ATL, DUP, FAB, HN, TRC, VPC.
Direct Yellow 103	ACS.
*Direct Yellow 105	ALT, GAF, HN, TRC.
*Direct Yellow 106	ACS, ALT, FAB, GAF, HN, TRC.
Direct Yellow 107	GAF, TRC.
Direct Yellow 114	ACY.
Direct Yellow 117	TRC.
Direct Yellow 118	TRC.
Direct Yellow 119	DUP.
Direct Yellow 120	DUP.
Direct Yellow 121	TRC.
Direct Yellow 123	DUP.
Direct Yellow 125	ACY.
Direct Yellow 127	DUP, TRC.
Direct Yellow 131	DUP.
Direct Yellow 132	VPC.
Other direct yellow dyes	AAP, ALT, ATL, GAF, HN, HSH, TRC, VPC.
*Direct orange dyes:	
*Direct Orange 1	AAP, ACS, ALT, ATL, BDO, CMG, VPC.
Direct Orange 6	ACS.
*Direct Orange 8	ACS, ATL, DUP, GAF, TRC, YAW.
Direct Orange 10	AAP.
Direct Orange 11	GAF.
*Direct Orange 15	ACS, ACY, DUP, GAF, HN, TRC.
*Direct Orange 26	ACS, ATL, CMG, GAF, HSH, TRC.
*Direct Orange 29	ATL, FAB, HN, TRC, VPC.
*Direct Orange 34	ACS, ATL, CMG, DUP, GAF.
*Direct Orange 37	ACY, ATL, CMG, DUP, GAF.
*Direct Orange 39	ACY, ALT, ATL, CMG, DUP, FAB, GAF, HN.
Direct Orange 59	DUP, GAF.
Direct Orange 61	TRC.
Direct Orange 67	ACS, VPC.
*Direct Orange 72	ACS, ATL, FAB, HN, HSH, TRC, VPC.
*Direct Orange 73	DUP, GAF, TRC, VPC.
Direct Orange 74	DUP, HSH.
Direct Orange 78	VPC.
Direct Orange 79	DUP.
Direct Orange 80	VPC.
Direct Orange 81	DUP, GAF, VPC.
Direct Orange 83	GAF.
Direct Orange 88	DUP.
*Direct Orange 102	ACS, ACY, ATL, DUP, GAF.
Direct Orange 110	TRC.
Other direct orange dyes	ALT, DUP, VPC.
*Direct red dyes:	
*Direct Red 1	ACS, ATL, DUP, GAF, TRC, VPC, YAW.
*Direct Red 2	ACS, ATL, DUP, FAB, HN, TRC.
*Direct Red 4	ACS, ATL, TRC, VPC.
Direct Red 5	ACS.
Direct Red 7	ATL.
*Direct Red 10	AAP, ACS, ATL, YAW.
*Direct Red 13	ACS, ATL, DUP, GAF, TRC, YAW.
Direct Red 16	ACS, ATL, DUP, TRC.
Direct Red 20	ACS, ATL, GAF.
*Direct Red 23	ACS, ATL, CMG, DUP, FAB, GAF, HN, TRC, VPC.
*Direct Red 24	AAP, ACS, ATL, FAB, HN, HSH, TRC, VPC.
*Direct Red 26	ACS, ATL, CMG, DUP, FAB, GAF, HN, HSH, TRC, VPC.
*Direct Red 28	ACS, ATL, DUP, TRC, YAW.
*Direct Red 31	ACS, ATL, DUP, GAF, HSH, TRC.
Direct Red 32	ACS.
*Direct Red 37	ACS, ATL, DUP, GAF, TRC, YAW.

TABLE 4. --DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1974--CONTINUED

Dye	Manufacturers' identification codes (according to list in table 3)
DIRECT DYES--Continued	
*Direct red dyes--Continued	
*Direct Red 39	ATL, DUP, GAF, TRC, YAW.
Direct Red 46	ATL.
Direct Red 62	ATL, TRC.
*Direct Red 72	ACS, DUP, GAF, TRC.
Direct Red 73	ACS, ATL.
*Direct Red 75	ACS, ATL, CMG, GAF.
Direct Red 76	GAF.
*Direct Red 79	ATL, CMG, HN, TRC, VPC.
*Direct Red 80	ACS, ALT, ATL, BDO, CMG, FAB, HN, HSH, SDH, TRC, VPC.
*Direct Red 81	ACS, ACY, ALT, ATL, CMG, DUP, GAF, HN, HSH, TRC, VPC, YAW.
*Direct Red 83	ACS, ALT, ATL, FAB, HN, HSH, TRC, VPC.
Direct Red 84	ATL.
Direct Red 95	VPC.
Direct Red 100	ATL.
Direct Red 111	GAF.
Direct Red 117	DUP.
Direct Red 120	CMG, VPC.
*Direct Red 122	ATL, CMG, TRC, VPC.
*Direct Red 123	ATL, CMG, GAF.
Direct 127 and 127A	ATL, CMG.
Direct Red 139	ATL, VPC.
Direct Red 149	ATL, CMG, DUP.
Direct Red 152	CMG.
Direct Red 153	ATL, CMG.
Direct Red 209	TRC, VPC.
Direct Red 212	VPC.
Direct Red 236	DUP.
Direct Red 238	DUP.
Other direct red dyes	ALT, ATL, GAF, HN, HSH, TRC.
*Direct violet dyes:	
Direct Violet 1	ACS, ATL.
*Direct Violet 7	ACS, ATL, GAF.
*Direct Violet 9	ACS, ATL, DUP, GAF, HN, TRC.
Direct Violet 14	ACS, ATL.
Direct Violet 22	DUP.
Direct Violet 47	GAF.
Direct Violet 48	ACS.
*Direct Violet 51	ACS, ATL, DUP.
Direct Violet 62	ACY.
Direct Violet 66	ATL, TRC.
Direct Violet 67	DUP.
Other direct violet dyes	ALT.
*Direct blue dyes:	
*Direct Blue 1	AAP, ACS, ACY, ATL, DUP, GAF, HN, TRC, VPC, YAW.
*Direct Blue 2	AAP, ACS, ATL, DUP, FAB, GAF, HN, HSH, TRC, VPC, YAW.
*Direct Blue 6	AAP, ACS, ACY, ATL, DUP, GAF, HN, HSH, TRC, YAW.
*Direct Blue 8	ACS, ALT, ATL, DUP, GAF.
Direct Blue 14	ACS, ATL, TRC.
*Direct Blue 15	ACS, ATL, DUP, GAF, VPC, YAW.
*Direct Blue 22	ACS, ATL, CMG.
*Direct Blue 24	ATL, HN, YAW.
*Direct Blue 25	ACS, ATL, GAF, TRC, YAW.
Direct Blue 26	ATL.
*Direct Blue 67	ACS, ATL, DUP, TRC.
*Direct Blue 71	ACS, ATL, GAF, TRC, VPC.
Direct Blue 74	DUP.
Direct Blue 75	TRC.
*Direct Blue 76	ACS, ALT, ATL, GAF, HN, HSH, TRC, VPC.
*Direct Blue 78	ACS, ATL, CMG, DUP, TRC.
*Direct Blue 80	ACS, ALT, ATL, DUP, FAB, GAF, HN, HSH, TRC, VPC.
Direct Blue 81	ATL.
*Direct Blue 86	AAP, ACS, ALT, ATL, DUP, FAB, GAF, HN, ICC, SDH, TRC, VPC

TABLE 4.--DYES FOR WHICH U.S PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971--CONTINUED

Dye	Manufacturers' identification codes (according to list in table 3)
DIRECT DYES--Continued	
*Direct blue dyes--Continued	
Direct Blue 87	ICI.
Direct Blue 91	TRC.
*Direct Blue 98	ATL, GAF, TRC, VPC.
Direct Blue 100	ALT, ATL, HN.
Direct Blue 104	DUP.
*Direct Blue 120, 120A	ATL, DUP, FAB, HN, TRC.
*Direct Blue 126	ATL, DUP, HSH, TRC, VPC.
Direct Blue 136	GAF.
Direct Blue 143	DUP.
Direct Blue 151	ATL, TRC.
Direct Blue 160	TRC.
Direct Blue 189	TRC.
Direct Blue 191	AAP, ACS, ALT, GAF.
Direct Blue 199	DUP, GAF, HN.
*Direct Blue 218	ACS, ALT, ATL, DUP, FAB, GAF, HN, TRC, VPC.
Direct Blue 224	ATL.
Direct Blue 238	ACY.
Direct Blue 263	DUP.
Other direct blue dyes	ALT, GAF, VPC.
*Direct green dyes:	
*Direct Green 1	AAP, ACS, ACY, ATL, FAB, GAF, TRC, YAW.
*Direct Green 6	AAP, ACS, ATL, FAB, GAF, HN, TRC, YAW.
Direct Green 8	TRC,
Direct Green 26	DUP, TRC.
Direct Green 27	DUP, TRC.
Direct Green 28	TRC.
Direct Green 38	DUP, GAF.
Direct Green 39	GAF.
Direct Green 45	ATL.
Direct Green 46	VPC.
Direct Green 47	ATL, DUP, GAF.
Direct Green 51	TRC.
Direct Green 69	TRC.
Other direct green dyes	ACY, ALT, DUP.
*Direct brown dyes:	
Direct Brown 1	ACY, ATL, HN.
*Direct Brown 1A	GAF, TRC, YAW.
Direct Brown 2	AAP, ACS, ACY, ATL, DUP, GAF, HN, HSH, TRC, YAW.
Direct Brown 3	VPC.
Direct Brown 6	TRC, YAW.
*Direct Brown 31	AAP, ACS, ATL, DUP, GAF, TRC, YAW.
Direct Brown 32	GAF.
Direct Brown 33	DUP.
Direct Brown 40	AAP.
Direct Brown 44	GAF, YAW.
Direct Brown 48	AAP.
Direct Brown 59	YAW.
*Direct Brown 74	AAP, ACS, DUP.
*Direct Brown 95	ACS, ATL, DUP, FAB, GAF, HN, HSH, TRC, YAW.
Direct Brown 106	GAF.
*Direct Brown 111	DUP, GAF, TRC.
Direct Brown 112	ATL.
*Direct Brown 154	ACS, DUP, FAB, TRC, YAW.
Direct Brown 218	ACS.
Other direct brown dyes	ALT, ATL, HN, HSH, VPC.
*Direct black dyes:	
*Direct Black 4	ACS, ATL, GAF, HN, TRC, YAW.
Direct Black 8	TRC, YAW.
*Direct Black 9	ACS, ATL, DUP, HN.
Direct Black 17	GAF.
*Direct Black 19	ATL, GAF, HN, TRC.
*Direct Black 22	ALT, ATL, GAF, HN, TRC, VPC, YAW.
Direct Black 36	AAP.

TABLE 4. --DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971--CONTINUED

Dye	Manufacturers' identification codes (according to list in table 3)
DIRECT DYES--Continued	
*Direct Black dyes--Continued	
Direct Black 37	AAP.
*Direct Black 38	ACS, ACY, FAB, GAF, HN, HSH, TRC, YAW.
Direct Black 44	TRC.
*Direct Black 51	AAP, ACS, DUP, GAF, TRC.
Direct Black 56	ACS, TRC.
Direct Black 71	ATL.
Direct Black 75	GAF.
Direct Black 78	ACS, HN.
*Direct Black 80	AAP, ACS, ATL, FAB, HN, HSH, TRC, YAW.
Direct Black 190	ACS, HN, TRC.
Other direct black dyes	ACY, ALT, ATL, HSH, TRC, YAW.
DISPERSE DYES	
*Disperse yellow dyes:	
Disperse Yellow 1	GAF, ICI.
Disperse Yellow 2	DUP.
*Disperse Yellow 3	AAP, ALT, DUP, GAF, HN, HSH, ICC, TRC.
Disperse Yellow 5	GAF, HN, ICC.
Disperse Yellow 8	TRC.
*Disperse Yellow 23	AAP, ALT, DUP, EKT, GAF, HN, ICC, TRC.
Disperse Yellow 31	GAF.
Disperse Yellow 32	DUP.
*Disperse Yellow 33	AAP, EKT, GAF, ICC, TRC.
*Disperse Yellow 34	AAP, EKT, ICC.
*Disperse Yellow 42	AAP, ALT, BUC, DUP, EKT, GAF, HN, ICC, MAY, SDC, TRC.
Disperse Yellow 50	TRC.
*Disperse Yellow 54	AAP, DUP, GAF, ICC, SDC, TRC.
Disperse Yellow 58	HST.
Disperse Yellow 63	HST.
Disperse Yellow 67	DUP.
Disperse Yellow 68	HST.
Disperse Yellow 69	ACY.
Disperse Yellow 74	VPC.
Disperse Yellow 77	VPC.
Disperse Yellow 85	EKT.
Disperse Yellow 86	AAP, EKT.
Disperse Yellow 87	EKT.
Disperse Yellow 88	EKT.
Disperse Yellow 89	EKT.
Disperse Yellow 93	VPC.
Disperse Yellow 95	VPC.
Disperse Yellow 96	VPC.
Disperse Yellow 118	AAP.
Disperse Yellow 125	SDC.
Other disperse yellow dyes	EKT, MAY, SDC, TRC, VPC.
*Disperse orange dyes:	
*Disperse Orange 3	AAP, DUP, GAF, HN, HSH ICC, TRC.
*Disperse Orange 5	AAP, BUC, EKT, GAF, ICC, SDC.
Disperse Orange 13	HST.
Disperse Orange 16	AAP.
*Disperse Orange 17	AAP, EKT, GAF, HN, HSH, ICC.
Disperse Orange 21	TRC.
*Disperse Orange 25	DUP, EKT, HN, TRC.
Disperse Orange 28	AAP.
Disperse Orange 29	AAP, GAF.
Disperse Orange 30	ICC, TRC.
Disperse Orange 33	ALT, HST.
Disperse Orange 37	TRC.
Disperse Orange 38	TRC.
Disperse Orange 41	DUP.
Disperse Orange 44	DUP.
Disperse Orange 57	EKT.

TABLE 4.--DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971--CONTINUED

Dye	Manufacturers' identification codes (according to list in table 3)
DISPERSE DYES--Continued	
*Disperse orange dyes--Continued	
Disperse Orange 58	AAP, EKT.
Disperse Orange 59	EKT, ICC.
Disperse Orange 62	BUC, DUP.
Disperse Orange 65	VPC.
Disperse Orange 75	DUP.
Disperse Orange 78	TRC.
Disperse Orange 89	AAP.
Disperse Orange 90	AAP.
Disperse Orange 94	SDC.
Other disperse orange dyes	AAP, ALT, ATL, EKT, GAF, MAY, SDC, VPC.
*Disperse red dyes:	
*Disperse Red 1	AAP, DUP, DKT, GAF, HN, HSH, ICC, TRC.
Disperse Red 4	GAF, ICC, TRC.
*Disperse Red 5	AAP, EKT, GAF, HSH, ICC.
Disperse Red 7	AAP, GAF.
Disperse Red 9	ATL.
*Disperse Red 11	AAP, DUP, GAF, ICC.
*Disperse Red 13	AAP, DUP, GAF, ICC.
*Disperse Red 15	GAF, HSH, ICC, TRC.
*Disperse Red 17	AAP, DUP, EKT, GAF, ICC, TRC.
Disperse Red 30	EKT, TRC.
Disperse Red 31	ICC.
Disperse Red 35	EKT.
Disperse Red 54	ICC.
*Disperse Red 55	AAP, DUP, GAF, HN, TRC.
Disperse Red 56	DUP.
Disperse Red 59	ACY, DUP, GAF.
*Disperse Red 60	AAP, ALT, ATL, DUP, EKT, GAF, HN, SDC, TRC, VPC.
*Disperse Red 65	DUP, EKT, ICC, TRC.
Disperse Red 66	AAP.
Disperse Red 73	TRC.
Disperse Red 78	ICC, TRC.
Disperse Red 82	VPC.
Disperse Red 86	EKT, GAF.
Disperse Red 88	EKT.
Disperse Red 90	VPC.
Disperse Red 96	ACY.
Disperse Red 117	EKT.
Disperse Red 133	VPC.
Disperse Red 136	EKT.
Disperse Red 137	EKT.
Disperse Red 138	EKT.
Disperse Red 140	DUP.
Disperse Red 159	VPC.
Disperse Red 161	DUP.
Disperse Red 167	GAF.
Disperse Red 176	ICC.
Disperse Red 177	ICC.
Disperse Red 178	ICC.
Disperse Red 179	ICC.
Disperse Red 180	ICC.
Other disperse red dyes	AAP, ATL, DUP, EKT, GAF, ICC, MAY, SDC, TRC, VPC.
*Disperse violet dyes:	
*Disperse Violet 1	AAP, GAF, HSH, ICC, TRC.
*Disperse Violet 4	AAP, GAF, ICC.
Disperse Violet 8	GAF.
Disperse Violet 17	DUP.
Disperse Violet 18	DUP.
Disperse Violet 26	DUP.
*Disperse Violet 27	AAP, ACY, DUP, EKT, ICC, TRC.
Disperse Violet 28	TRC.
Disperse Violet 41	EKT.
Disperse Violet 42	EKT.
Disperse Violet 43	EKT.
Disperse Violet 44	EKT.
Other disperse violet dyes	GAF, MAY, SDC.

TABLE 4.--DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971--CONTINUED

Dye	Manufacturers' identification codes (according to list in table 3)
DISPERSE DYES--Continued	
*Disperse blue dyes:	
*Disperse Blue 1	AAP, BAS, GAF, ICC, TRC.
*Disperse Blue 3	AAP, DUP, EKT, GAF, HN, HSH, ICC, TRC.
*Disperse Blue 7	EKT, GAF, HN, HSH, ICC, TRC.
Disperse Blue 9	GAF, ICC.
Disperse Blue 27	EKT, TRC.
Disperse Blue 35	ICI.
Disperse Blue 54	ICC.
Disperse Blue 55	TRC.
Disperse Blue 56	VPC.
Disperse Blue 59	TRC.
Disperse Blue 60	DUP.
Disperse Blue 61	DUP.
Disperse Blue 62	DUP, GAF, SDC.
Disperse Blue 63	DUP.
*Disperse Blue 64	DUP, EKT, GAF, TRC.
Disperse Blue 70	AAP.
Disperse Blue 71	VPC.
Disperse Blue 72	ICI.
Disperse Blue 73	TRC.
*Disperse Blue 79	AAP, EKT, TRC.
Disperse Blue 81	VPC.
Disperse Blue 85	TRC.
Disperse Blue 94	BAS.
Disperse Blue 95	GAF.
Disperse Blue 102	EKT.
Disperse Blue 109	DUP.
Disperse Blue 112	EKT.
Disperse Blue 117	EKT.
Disperse Blue 118	EKT.
Disperse Blue 119	EKT.
Disperse Blue 120	EKT, GAF.
Disperse Blue 121	EKT.
Disperse Blue 123	EKT.
Disperse Blue 125	TRC.
Disperse Blue 133	DUP.
Disperse Blue 139	VPC.
Disperse Blue 150	DUP.
Disperse Blue 152	HST.
Disperse Blue 155	GAF.
Disperse Blue 166	ICC.
Other disperse blue dyes	ALT, ATL, DUP, EKT, GAF, HN, HSH, ICC, MAY, SDC, TRC, VPC.
Disperse green dyes	GAF, TRC, VPC.
Disperse brown dyes:	
Disperse Brown 1	TRC.
*Disperse Brown 2	DUP, EKT, GAF.
Disperse Brown 7	EKT.
Disperse Brown 8	VPC.
Disperse Brown 11	AAP.
Other disperse brown dyes	GAF, ICC, SDC.
*Disperse black dyes:	
*Disperse Black 1	AAP, DUP, GAF, TRC.
Disperse Black 2	ATL, TRC.
Disperse Black 9	AAP, EKT.
Disperse Black 33	EKT.
Disperse Black 34	EKT.
Other disperse black dyes	ALT, ATL, DUP, GAF, ICC, SDC, VPC.
FIBER-REACTIVE DYES	
*Reactive yellow dyes:	
Reactive Yellow 1	HST, ICI.
Reactive Yellow 2	TRC.
Reactive Yellow 3	TRC.
Reactive Yellow 4	HST, ICI.

TABLE 4.--DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971--CONTINUED

Dye	Manufacturers' identification codes (according to list in table 5)
FIBER-REACTIVE DYES--Continued	
*Reactive yellow dyes--Continued	
Reactive Yellow 6	HST, TRC.
Reactive Yellow 7	HST, ICI.
Reactive Yellow 13	HST.
Reactive Yellow 18	ICI.
Reactive Yellow 22	ICI.
Reactive Yellow 25	VPC.
Reactive Yellow 31	HST.
Reactive Yellow 37	HST.
Reactive Yellow 60	ACY.
Reactive Yellow 61	ACY.
Reactive Yellow 62	ACY.
Other reactive yellow dyes	HST.
Reactive orange dyes:	
Reactive Orange 1	ICI.
Reactive Orange 4	ICI.
Reactive Orange 5	TRC.
Reactive Orange 12	ICI.
Reactive Orange 13	ICI.
Reactive Orange 14	ICI.
Reactive Orange 16	HST.
Reactive Orange 50	HST.
Other reactive orange dyes	HST.
Reactive red dyes:	
Reactive Red 1	ICI.
Reactive Red 2	ICI.
Reactive Red 4	TRC.
Reactive Red 5	ICI.
Reactive Red 8	ICI.
Reactive Red 11	ICI, TRC.
Reactive Red 21	HST.
Reactive Red 29	ICI.
Reactive Red 31	ICI.
Reactive Red 33	ICI.
Reactive Red 35	HST.
Reactive Red 40	VPC.
Reactive Red 41	VPC.
Reactive Red 58	ICI.
Reactive Red 92	ACY.
Reactive Red 93	ACY.
Reactive Red 94	HST.
Reactive violet dyes:	
Reactive Violet 1	ICI.
Reactive Violet 2	TRC, VPC.
Reactive Violet 4	HST.
Reactive Violet 5	HST.
Other reactive violet dyes	HST.
*Reactive blue dyes:	
Reactive Blue 1	ICI.
Reactive Blue 2	TRC.
Reactive Blue 3	ICI.
Reactive Blue 4	ICI.
Reactive Blue 5	ICI, TRC.
Reactive Blue 7	TRC.
Reactive Blue 9	ICI.
Reactive Blue 19	HST.
Reactive Blue 20	HST.
Reactive Blue 21	HST.
Reactive Blue 25	ICI.
Reactive Blue 29	VPC.
Reactive Blue 30	VPC.
Reactive Blue 38	HST.
Reactive Blue 86	ACY.
Reactive Blue 87	ACY.
Reactive Blue 88	ACY.
Reactive Blue 89	HST.
Reactive Blue 90	HST.

TABLE 4.--DYES [illegible] IDENTIFIED BY [illegible]

Dye	Manufacturers' identification codes (according to list in table 3)
FIBER-REACTIVE DYES--Continued	
*Reactive blue dyes--Continued	
Reactive Blue 91	BAS.
Other reactive blue dyes	BAS, ICI.
Reactive green dyes:	
Reactive Green 6	BAS.
Reactive Green 20	HST.
Reactive brown dyes:	
Reactive Brown 10	[illegible]
Other reactive brown dyes	[illegible]
*Reactive black dyes	
Reactive Black 1	[illegible], TRC.
Reactive Black 5	[illegible]
FLUORESCENT BRIGHTENING AGENTS	
Fluorescent Brightening Agent 1	CGY.
Fluorescent Brightening Agent 6	ACY.
Fluorescent Brightening Agent 8	ACY.
Fluorescent Brightening Agent 9	GAF, SDH.
Fluorescent Brightening Agent 22	CGY.
Fluorescent Brightening Agent 24	CGY.
Fluorescent Brightening Agent 25	GAF.
Fluorescent Brightening Agent 28	ACY, CCW, DUP, SDH, VPC.
Fluorescent Brightening Agent 30	GAF.
Fluorescent Brightening Agent 33	GAF.
Fluorescent Brightening Agent 45	TRC.
Fluorescent Brightening Agent 46	CGY.
Fluorescent Brightening Agent 49	[illegible]
Fluorescent Brightening Agent 52	S.
Fluorescent Brightening Agent 54	CGY.
Fluorescent Brightening Agent 59	CGY.
Fluorescent Brightening Agent 61	ACY.
Fluorescent Brightening Agent 68	CCW, GAF.
Fluorescent Brightening Agent 71	ACY, GAF.
Fluorescent Brightening Agent 75	GAF.
Fluorescent Brightening Agent 102	DUP, VPC.
Fluorescent Brightening Agent 108	GAF.
Fluorescent Brightening Agent 109	GAF.
Fluorescent Brightening Agent 125	ACY.
Fluorescent Brightening Agent 126	SDH.
Fluorescent Brightening Agent 128	SDH.
Fluorescent Brightening Agent 130	CGY.
Fluorescent Brightening Agent 134	[illegible]
Fluorescent Brightening Agent 136	[illegible]
Fluorescent Brightening Agent 139	[illegible]
Fluorescent Brightening Agent 158	ACY.
Fluorescent Brightening Agent 159	ACY.
Fluorescent Brightening Agent 189	[illegible]
Other fluorescent brightening agents	[illegible], CCW, CGY, GAF, FCW, S, VPC.
FOOD, DRUG, AND COSMETIC COLORS	
*Food, Drug, and Cosmetic Dyes	
*FD&C Blue No. 1	[illegible], ALT, KON, SDH, WJ.
*FD&C Blue No. 2	[illegible], ALT, KON, SDH, WJ.
FD&C Green No. 3	[illegible], WJ.
*FD&C Red No. 2	[illegible], ALT, KON, SDH, STG, WJ.
*FD&C Red No. 3	[illegible], ALT, KON, SDH, STG, WJ.
FD&C Red No. 4	[illegible], KON, STG.
*FD&C Violet No. 1	[illegible], DH, WJ.
*FD&C Yellow No. 5	[illegible], ALT, KON, STG, WJ.
*FD&C Yellow No. 6	[illegible], ALT, KON, SDH, STG, WJ.
Other food, drug, and cosmetic dyes	[illegible].

TABLE 4.--DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971--CONTINUED

Dye	Manufacturers' identification codes (according to list in table 3)
FOOD, DRUG, AND COSMETIC COLORS--Continued	
Drug and Cosmetic Dyes	
D&C Blue No. 6	KON.
D&C Green No. 5	ACS, ALT, KON.
D&C Green No. 6	ACS, ALT, KON.
D&C Green No. 8	KON, SDH.
*D&C Orange No. 4	KON, SNA, TMS.
D&C Orange No. 5	SNA, TMS.
D&C Orange No. 10	TMS.
D&C Orange No. 17	SNA.
D&C Red No. 2	KON.
D&C Red No. 3	KON.
*D&C Red No. 6	KON, SNA, TMS.
*D&C Red No. 7	KON, SNA, TMS.
D&C Red No. 8	KON, SNA.
*D&C Red No. 9	KON, SNA, TMS.
D&C Red No. 10	KON, SNA.
D&C Red No. 11	KON, SNA.
D&C Red No. 12	SNA, TMS.
D&C Red No. 13	SNA, TMS.
D&C Red No. 17	KON.
*D&C Red No. 19	ACS, KON, SNA, TMS.
*D&C Red No. 21	KON, SNA, TMS.
D&C Red No. 22	KON.
D&C Red No. 27	SDH, SNA, TMS
D&C Red No. 28	ACS, TMS.
D&C Red No. 30	KON, TMS.
D&C Red No. 31	KON.
D&C Red No. 33	ACS, KON.
D&C Red No. 34	KON.
*D&C Red No. 36	ALT, KON, TMS.
D&C Red No. 37	ACS.
D&C Yellow No. 5	KON, TMS.
D&C Yellow No. 6	KON.
D&C Yellow No. 7	ALT, KON.
D&C Yellow No. 8	KON, TMS.
D&C Yellow No. 10	KON.
D&C Yellow No. 11	KON.
Drug and Cosmetic Dyes, External	
Ext. D&C Green No. 1	ACS, KON.
Ext. D&C Yellow No. 1	ACS, KON.
Ext. D&C Yellow No. 7	KON.
INGRAIN DYES	
Ingrain blue dyes:	
Ingrain Blue 1	ICI.
Ingrain Blue 3	ICI.
MORDANT DYES	
*Mordant yellow dyes:	
*Mordant Yellow 1	ATL, GAF, PDC.
Mordant Yellow 3	ATL.
Mordant Yellow 5	TRC.
*Mordant Yellow 8	ACS, PDC, VPC.
Mordant Yellow 14	ACS.
Mordant Yellow 16	ACY.
Mordant Yellow 20	ACS, ATL.
Mordant Yellow 26	VPC.
Mordant Yellow 29	GAF.
Mordant Yellow 30	TRC, VPC.

TABLE 4.--DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971--CONTINUED

Dye	Manufacturers' identification codes (according to list in table 3)
MORDANT DYES--Continued	
*Mordant orange dyes:	
Mordant Orange 1	ACY, PDC, TRC.
Mordant Orange 4	GAF.
Mordant Orange 6	ATL, GAF, PDC, TRC.
Mordant Orange 8	TRC.
*Mordant red dyes:	
Mordant Red 3	ACY.
Mordant Red 5	PDC.
Mordant Red 7	ACY, ATL, BDO, CMG, GAF, PDC, TRC, VPC.
Mordant Red 9	MRX.
Mordant Red 11	ACY.
Mordant Red 64	PDC.
Mordant violet dyes:	
Mordant Violet 5	PDC.
Mordant Violet 11	GAF.
Mordant blue dyes:	
Mordant Blue 1	GAF.
Mordant Blue 3	GAF.
Mordant Blue 9	GAF.
Mordant Blue 13	ACS.
Mordant Blue 19	CMG.
Mordant green dyes:	
Mordant Green 11	ACY.
Mordant Green 36	PDC.
Mordant brown dyes:	
*Mordant Brown 1	ACS, CMG, DUP, GAF, TRC, YAW.
Mordant Brown 12	PDC.
Mordant Brown 13	ACS.
Mordant Brown 15	GAF.
Mordant Brown 18	ACS, DUP.
Mordant Brown 19	GAF.
Mordant Brown 21	GAF, VPC.
*Mordant Brown 33	ACS, GAF, PDC, TRC.
*Mordant Brown 40	ACS, CMG, GAF, VPC, YAW.
Mordant Brown 50	TRC.
Mordant Brown 63	TRC.
Mordant Brown 70	DUP, PDC.
*Mordant black dyes:	
Mordant Black 1	ACS.
Mordant Black 3	ACS, TRC.
Mordant Black 7	GAF.
Mordant Black 8	VPC.
Mordant Black 9	ACS, VPC.
*Mordant Black 11	ACS, GAF, TRC, VPC.
Mordant Black 13	HSH.
*Mordant Black 17	ACS, ACY, GAF, TRC.
Mordant Black 19	PDC.
Mordant Black 26	TRC.
OXIDATION BASES	
Oxidation Base 8 and 8A	ACY.
Oxidation Base 21	PDC.
Oxidation Base 22	ACY.
Oxidation Base 25	ACY.
Other oxidation bases	ACY, CMG.
SOLVENT DYES	
*Solvent yellow dyes:	
Solvent Yellow 1	AAP.
*Solvent Yellow 2	AAP, DUP, GAF, PSC.
Solvent Yellow 3	ACS, PSC.
Solvent Yellow 13	ACY, GAF, PSC.

TABLE 4.--DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971--CONTINUED

Dye	Manufaccurers' identification codes (according to list in table 3)
SOLVENT DYES--Continued	
*Solvent yellow dyes--Continued	
*Solvent Yellow 14	AAP, ACS, ACY, DUP, GAF, PSC.
Solvent Yellow 19	GAF.
Solvent Yellow 29	GAF.
Solvent Yellow 30	ACS.
Solvent Yellow 33	AAP, ACS, ACY.
Solvent Yellow 34	ACY, DSC.
Solvent Yellow 40	ACS.
Solvent Yellow 42	ACS.
Solvent Yellow 43	GAF.
Solvent Yellow 44	ACS, GAF.
Solvent Yellow 45	ACS.
*Solvent Yellow 47	ACY, DUP, GAF.
Solvent Yellow 56	ACS, ACY.
Solvent Yellow 71	ACY.
Solvent Yellow 72	ACY.
Solvent Yellow 87	ACY.
Other solvent yellow dyes	AAP, ATL, DSC, PAT.
*Solvent orange dyes:	
Solvent Orange 2	AAP, PSC.
*Solvent Orange 3	ACS, ACY, DSC, GAF, PSC.
Solvent Orange 5	GAF.
*Solvent Orange 7	ACS, ACY, GAF.
Solvent Orange 20	ACY, GAF.
Solvent Orange 23	ACS.
Solvent Orange 24	DUP.
Solvent Orange 25	ACY, DUP.
Solvent Orange 31	ACS.
Solvent Orange 48	ACY.
Solvent Orange 51	ACY.
Other solvent orange dyes	AAP, ACY, DSC, DUP, PAT.
*Solvent red dyes:	
Solvent Red 1	PSC.
Solvent Red 8	GAF.
Solvent Red 22	GAF.
Solvent Red 24	ACY, DUP, GAF.
*Solvent Red 26	AAP, ACS, ACY, PSC.
Solvent Red 27	ACS.
Solvent Red 33	DUP, GAF.
Solvent Red 35	GAF.
Solvent Red 40	GAF.
Solvent Red 41	DSC.
*Solvent Red 49	ACY, DSC, DUP, GAF.
Solvent Red 52	AAP, GAF, ICI.
Solvent Red 68	ACS.
Solvent Red 69	DSC, DUP.
Solvent Red 74	ACS.
Solvent Red 75	ACS.
Solvent Red 105	ACY.
Solvent Red 108	ACY.
Solvent Red 111	ACY.
Solvent Red 115	ACY.
Solvent Red 126	ACY.
Other solvent red dyes	AAP ACY, ATL, DSC, DUP, ICI. PAT.
*Solvent violet dyes:	
Solvent Violet 8	ACY. DSC.
Solvent Violet 9	DSC
Solvent Violet 13	AAP, ATL, HSH, ICI.
Solvent Violet 14	AAP, ICI.
Other solvent violet dyes	AAP, DSC, PAT.
Solvent blue dyes:	
Solvent Blue 3	ACY, SW.
Solvent Blue 4	DSC, DUP, SDH.
Solvent Blue 5	DSC.

TABLE 4.--DYES FOR WHICH U.S. PRODUCTION OR SALES WERE REPORTED, IDENTIFIED BY MANUFACTURER, 1971--CONTINUED

Dye	Manufacturers' identification codes (according to list in table 3)
SOLVENT DYES--Continued	
Solvent blue dyes -Continued	
Solvent Blue 6	DSC.
Solvent Blue 7	ACY.
Solvent Blue 9	GAF.
Solvent Blue 11	BDO, GAF, ICI.
Solvent Blue 12	BDO.
Solvent Blue 16	ACS.
Solvent Blue 36	ACS, DUP.
Solvent Blue 37	DUP.
*Solvent Blue 38	ACS, ACY, ATL, DUP, GAF.
Solvent Blue 43	ACS.
Solvent Blue 57	DUP.
Solvent Blue 58	ACY.
Solvent Blue 59	ACY.
Solvent Blue 60	ACY.
Other solvent blue dyes	AAP, ACY, DSC, GAF, ICI, PAT, x.
Solvent green dyes:	
Solvent Green 1	ACY, DSC.
Solvent Green 2	GAF.
*Solvent Green 3	AAP, ACS, ACY, ATL, GAF, HSH, ICI.
Other solvent green dyes	ACY, DSC, GAF.
Solvent brown dyes:	
Solvent Brown 11	GAF.
*Solvent Brown 12	ACY, DSC, GAF.
Solvent Brown 19	DUP.
Solvent Brown 20	ACY, DUP.
Solvent Brown 22	DUP, PSC.
Solvent Brown 38	ACY.
Other solvent brown dyes	DSC.
Solvent black dyes:	
Solvent Black 3	ACS.
Solvent Black 5	ACS, ACY, DSC, DUP.
Solvent Black 7	ACS, ACY, DSC.
Solvent Black 12	ACS.
Solvent Black 13	ACS.
Solvent Black 17	DUP.
Solvent Black 26	ACY.
Other solvent black dyes	ATL, DSC, GAF.

Table 5. Azo Dyes Produced in 1971 but Not in 1972

Acid Yellow	77	ACY	Disperse Orange	13	HST
Orange	4	ACY	Orange	28	AAP
Red	42	GAF	Red	56	duP
Red	100	VPC			
Blue	10	AAP,ACS	Reactive Blue	9	ICI
Blue	89	ACS	Blue	20	HST
Blue	161	VPC	Black	1	HST,TRC
Black	138	VPC			
			Mordant Yellow	3	ATL
Basic Yellow	26	ACY	Red	5	PDC
Red	23	VPC	Blue	13	ACS
			Green	11	ACY
Direct Yellow	9	ATL	Brown	50	TRC
Red	5	ACS	Black	1	ACS
Red	46	ATL	Black	7	GAF
Violet	1	ACS,ATL			
Blue	238	ACY			
Green	46	VPC			
Brown	3	VPC			
Brown	33	duP			

Table 6. Azo Dyes Produced in 1972 but Not in 1971

Acid	Yellow	4	SDH	Reactive	Yellow	15	HST
	Orange	5	ACY		Yellow	17	HST
	Red	179	TRC		Black	5	HST
	Red	277	VPC				
	Black	53	PSC	FD&C	Red	40	ACS,WJ
	Black	139	VPC	D&C	Red	39	SDH
Disperse	Red	21	EKT	Mordant	Yellow	36	PDC

TABLE 7.--DYES: DIRECTORY OF MANUFACTURERS, 1971

ALPHABETICAL DIRECTORY BY CODE

[Names of dye manufacturers that reported production or sales to the U.S. Tariff Commission for 1971 are listed below in the order of their identification codes as used in table 2]

Code	Name of company
AAP	American Aniline Products, Inc.
ACS	Allied Chemical Corp., Specialty Chemicals Div.
ACY	American Cyanamid Co.
ALL	Alliance Chemical, Inc.
ALT	Crompton & Knowles Corp., Althouse Div.
ATL	Atlantic Chemical Corp.
BAS	BASF Wyandotte Corp.
BDO	Benzenoid Organics, Inc.
BUC	Blackman-Uhler Chemical Co.
CCW	Cincinnati Malacron Chemicals, Inc.
CGY	Ciba-Geigy Corp.
CNG	Nyanza, Inc.
CPC	Childs Pulp Colors, Inc.
CWN	Upjohn Co., Fine Chemical Div.
DSC	Dye Specialties, Inc.
DUP	E. I. duPont de Nemours & Co., Inc.
EKT	Eastman Kodak Co., Tennessee Eastman Co., Div.
FAB	Fabricolor Manufacturing Corp.
GAF	GAF Corp., Chemical Div.
HN	Tenneco Chemicals, Inc.
HSC	Chemetron Corp., Pigments Div.
HSH	Harshaw Chemical Co. Div. of Kewanee Oil Co.
HST	American Hoechst Corp.
ICC	Inmont Corp.
ICI	ICI America, Inc
KON	H. Kohnstamm & Co., Inc.
MAY	Otto B. May, Inc.
MRX	Max Marx Color & Chemical Co
PAT	Morton International, Inc., Morton Chemical Co. Div.
PCW	Pfister Chemical Works
PDC	Berncolors-Poughkeepsie, Inc.
PSC	Passaic Color & Chemical Co.
S	Sandoz, Inc., Sandoz Color & Chemicals Div.
SDC	Martin-Marietta Corp., Southern Dyestuff Co. Div.
SDH	Sterling Drug, Inc., Hilton-Davis Chemical Co. Div.
SNA	Sun Chemical Corp.
STC	Sou-Tex Chemical Co., Inc.
STG	Stange Co.
SW	Sherwin-Williams Co.
TMS	Sterling Drug, Inc., Thomasset Colors Div.
TRC	Toms River Chemical Corp.
VPC	Verona Corp.
WJ	Warner-Jenkinson Manufacturing Co.
YAW	Y.S. Young, Young Aniline Works Div.

Note.--Complete names and address of the above reporting companies, will be listed in the Tariff Commission's annual report, Synthetic Organic Chemicals, United States Production and Sales, 1971.

SUMMARY AND CONCLUSION AS TO DEGREE OF HAZARD

Only two bromohydrocarbons, methyl bromide and ethylene dibromide, are known to be in large scale production and use. Most of the methyl bromide is used in the fumigation of stored agricultural products and soil sterilization at an annual increase of about 10%. Most of the ethylene dibromide is used in leaded gasoline as a scavenger for lead deposits in engines; perhaps an amount equivalent to that of methyl bromide is used for the same purposes. Production is expected to decrease with the decreased usage of leaded gas.

Both react to some extent, especially the methyl bromide, with protein in the foods they contact. Resultant toxicity or reduction in nutritional values is thought to be of little concern to humans or animals fed foodstuffs which have been given sufficient time to allow residual, unreacted fumigant to evaporate. Egg size and quantity may be reduced in poultry fed too much ethylene dibromide as residue in the feed.

A variety of metabolites comprises the urinary pathway of excretion of bromohydrocarbons. These include alkyl and hydroxyalkyl mercapturic acids and S-oxide mercapturic acids, similarly changed peptides, etc. Complete breakdown to carbon dioxide has also been demonstrated.

Methyl bromide is a very toxic substance with many known fatalities from occupational use. Its low detectability by human senses at fatal air concentrations is especially dangerous to dock and warehouse workers who may not have been informed that a cargo or shipment was recently fumigated. Death from an acute dose usually results from lung damage, but kidney damage is also immediate. Chronic exposure can produce brain and spinal cord damage, occasionally with effects lasting long after cessation of exposure.

Ethylene dibromide is rated as a highly toxic substance, but its relatively low volatility is probably the contributing factor in a lack of reported fatalities connected with its use.

Neither compound appears to offer an environmental threat, there being no indication of accumulation in laboratory animals given extraordinary doses, and there being no indication of effective soil accumulation from annual application.

BROMINATED HYDROCARBONS

I. PHYSICAL PROPERTIES

Some appropriate physical properties of many of the C_1-C_3 mono- and polybromohydrocarbons are given in Table 1. The references used, Dow Chemical and Sax, provide similar information on many other bromohydrocarbons of considerably lesser economic or toxicological importance but frequently used in research laboratories. The compounds in the table should be considered soluble in a variety of organic solvents and, at best, slightly soluble in water. Tetrabromomethane, allyl bromide and propargyl bromide are lachrymatory. The vapor pressure of ethyl bromide is 400 mm Hg at 21°C, that of ethylene dibromide is 17.4 mm Hg at 30°C, and that of methyl bromide is 1420 mm Hg at 20°C.

Hassall (1953) reported the following saturation vapor pressures at 25° in mm Hg: ethyl bromide, 468; propyl bromide, 135; butyl bromide, 38.9; amyl bromide, 13.8; hexyl bromide, 10.

Saracco and Marchetti (1958) provided the following equation for estimating the water solubility of bromohydrocarbons (straight chain): $\ln S = \ln S_o - Kn$, in which S is in units of moles/liter, So has the value 1.63, K has the value 1.46, and n is the number of carbon atoms in the chain.

Hill (1962) studied the explosive limits range of air-methyl bromide mixtures as a function of pressure, and found that increases in pressure over atmospheric allowed mixtures relatively rich in methyl bromide to explode. For example his range at one atmosphere was 10-15.4% methyl bromide, but at 8-9 atmospheres a 23% methyl bromide mix exploded, and at 6-7 atmospheres a 29.5% mix exploded.

Table 1. Properties of Some Bromohydrocarbons[a]

	Freezing Point[b]	Boiling Point[b]	Flash Point[b]	Fire Point[b]	Autoignition Point[b,c]	Expl. Limits[c]	Sp. Gravity,[d] Vapor density	Index of Refraction[e]
Methyl Bromide CH_3Br	-94.1	3.6	-	-	536	10-16%	1.746(-5)[f], 3.27	
Methylene Dibromide CH_2Br_2	-52	99	-	-			2.49	1.5381
Bromoform $CHBr_3$	7.8	148.9	-	-			2.88	1.5944
Tetrabromomethane CBr_4	92-3	189	-	-				
Ethyl Bromide CH_3CH_2Br	-119.3	38.4	-	-	511	6.7-11.3%	1.4492, 3.76	1.4210
Vinyl Bromide $CH_2=CHBr$	-139.2	15.8	-	-			1.549(10)[f]	1.4412(10)
Ethylene Dibromide $BrCH_2CH_2Br$	10	131.4	-	-			2.17, 6.48	1.5360
Acetylene Tetrabromide $Br_2CHCHBr_2$	-0.1	245.8 (dec.)	-	-			2.96	1.6350
Propyl Bromide $CH_3CH_2CH_2Br$	-109.9	71	-	-			1.350	1.4314
Allyl Bromide $CH_2=CHCH_2Br$	<-50	70.2	-1.1	32.2[g]			1.412	1.465
Propargyl Bromide $CH\equiv CCH_2Br$	-62	84.4	21.1	expl.			1.582	1.4912

a - from Dow Chemical Co. Bulletin 164-100-68 (1968)
b - in degrees centigrade
c - from Sax
d - at 25/25°
e - at 25°
f - density at (x°C)
g - open cup
- none

Forshey et al (1969) studied the fire and explosion potential of propargyl bromide. Vapors would propagate a flame in a 19-cm diameter container at a gauge pressure of 0.03 psia at room temperature. Accidental pressurization of the aerated liquid could ignite it.

II. PRODUCTION

The U.S. Tariff Commission Reports contained the following figures for production (in metric tons):

	Methyl Bromide	Ethyl Bromide	Ethylene Dibromide
1969	9,080	791	140,600
1970	9,540	-	134,800
1971	-	-	127,000
1972	11,160	-	143,100
1973	13,410	-	-

The April 1974 U.S.T.C. Preliminary Report on 1972 Miscellaneous Chemicals production listed the following brominated hydrocarbons and manufacturers:

1-bromobutane	Michigan Chemical Corp. (MCH)
2-bromobutane	Abbott Labs., Eastman Kodak Co. (EK)
bromoethane	Dow Chemical Co., Great Lakes Chemical Corp. (GTL), MCH
1-bromohexane	Humphrey Chemical Co.
1-bromo-3-methyl-butane	Eli Lilly & Co. (LIL)
1-bromo-3-methyl-2-butene	Sterling Drug, Inc. - Winthrop Labs. Div. (SDW)
1-bromo-octadecane	du Pont
1-bromo-octane	MCH

2-bromopentane	LIL
1-bromopropane	EK, SDW
1,2-dibromoethane	Dow, GTL, MCH, Pittsburgh Plate Glass Co., Ethyl Corp.
Dibromomethane	Dow

In the June 1974 Preliminary Report on Pesticides and Related Products for 1973 the following manufacturers are listed for methyl bromide: Kerr-McGee Chemical Corp., Dow, GTL, and MCH.

The Chemical Week Buyers Guide for 1974 lists the following compounds for sale:

acetylene tetrabromide	hexamethylene dibromide
allyl bromide	n-hexyl bromide
n-amyl bromide	methylene dibromide
i-amyl bromide	nonyl bromide
bromocyclohexane	octadecyl bromide
bromocyclopentane	octyl bromide
bromoform	pentamethylene bromide
i-butyl bromide	propylene dibromide
s-butyl bromide	tetrabromomethane
t-butyl bromide	tetradecyl bromide
n-decyl bromide	trimethylene dibromide
n-heptyl bromide	undecyl bromide
n-hexadecyl bromide	vinyl bromide

Product bulletins from Dow and White Chemical Corporation indicate that both can supply a wide variety of bromohydrocarbons.

III. USE

Ethylene dibromide, the largest tonnage bromohydrocarbon, is used mostly as a lead scavenger in leaded gasoline. It is difficult to project a future trend in this area because of the possibility of changes in the consumption of tetraalkyl lead in gasoline in connection with exhaust emission regulations and potential engine design changes. While it is likely that the new engines will use unleaded or lightly leaded gas, there will remain in existence for years millions of cars intended to be fueled with high lead gas. A relatively small amount of production is used as a fumigant for stored grain, for soil, as a dye and pharmaceutical intermediate, and as a solvent.

Methyl bromide has been used in the past as a fire extinguishing agent, under the tradename Halon 1001. Its extremely high toxicity, coupled with its tendency to corrode the usual metallic containers, ended this use. Petrella and Sellers (1970) compared Halon 1001 with a number of other Halons (mixed-halogen compounds of methane or ethane) in their relative fire extinguishing capabilities; their conclusion was that the toxicity of Halon 1001 far outweighed its superiority.

Most of the methyl bromide produced is used as a fumigant for stored agricultural products and as a sterilizing agent for soil. Its high volatility requires that an enclosure or impermeable cover be present to ensure complete and economic utilization. When used in buildings it is blended with 2% of chloropicrin (CCl_3NO_2), the lachrymatory action of which acts as a warning for the methyl bromide which is undetectable by human senses in deadly concentrations.

The major use for ethyl bromide, and a minor one for methyl bromide, is as an alkylating agent in drug manufacture. Lesser amounts of the ethyl are used as a solvent or refrigerant.

The following table on properties and uses of other bromohydrocarbons was adapted from one in Kirk-Othmer, Vol. 3, pp 776-8 (1964).

Table 2. Properties and Uses of Miscellaneous Bromohydrocarbons[a]

Compound	Mp, °C	Bp, °C	d(20/4°)	n_D^{20}	Use[b]
Acetylene tetrabromide					G,H,M, Solv
Allyl bromide					F, Syn
Bromoform					G,H,P, Syn
n-Butyl bromide $CH_3(CH_2)_2CH_2Br$	-112.7	100.5	1.2687(25/4°)	1.4398	Syn
Carbon tetrabromide			3.42	1.60 (99.5)	Bromination agent
Ethylidene bromide CH_3CHBr_2		109	2.06	1.5122	H, Syn
Isopropyl bromide $(CH_3)_2CHBr$	-89.0	59.3	1.3138	1.4254	Solv, Syn
Lauryl bromide $CH_3(CH_2)_{10}CH_2Br$		177 (45 mm)	1.0382	1.4581	Syn
Methylene bromide					E,G,H, Solv, Syn
Propargyl bromide					F, Syn
n-Propyl bromide $CH_3CH_2CH_2Br$	-110	70.9	1.3514	1.4341	Solv, Syn
Propylene bromide $CH_3CHBrCH_2Br$	-55.3	140	1.9333	1.5194	Solv, Syn
1,2,3-Tribromopropane $BrCH_2CHBrCH_2Br$	16	220	2.4076 (25/4°)	1.5835 (25)	H, Syn
Trimethylene bromide $BrCH_2CH_2CH_2Br$	-34.2	167.3	1.9790	1.5232	Syn of cyclopropane
Vinyl bromide					Copolymer

a - Properties of those compounds also in Table 1 are not reproduced
b - Explanation of letters and abbreviations is as follows:

E - Ingredient of fire-extinguishing fluids or as a fire retardant

F - Fumigant, if very volatile, or contact poison

G - Gage fluid

H - Heavy liquid for flotation-type ore separation

M - Microscopic or refractometric fluid

P - Ingredient of medicinal or pharmaceutical products

Solv - Solvent, generally for fats, waxes, or resins, possibly as a reaction medium

Syn - Intermediate in synthesis of other compounds

Barduhn et al (1960) examined methyl bromide and found it to be promising as a demineralizing agent for sea water because of the hydrate it forms under pressure.

Huang et al (1966) studied the concentration of fruit juices by the use of methyl bromide to remove some of the water as complexed crystals. While an effective concentration was achieved, some of the natural flavor was lost and an undesirable flavor added.

IV. CURRENT PRACTICE

ICC shipping regulations for liquid methyl bromide require poison and poison B labels, and limit the quantity to 208 liters (55 gal.) The Coast Guard requires poison, poison B, and MCA warning labels. The IATA does not allow it on passenger craft, but does allow 220 liters (58 gal.) on cargo craft with poison and poison B lables.

General regulations for ethyl bromide call for the MCA label; IATA gives it a Class A status, allowing 40 liters (10.6 gal.) on passenger, 220 liters (58 gals.) cargo crafts.

Ethylene dibromide must have the MCA label. It is required by IATA to have the poison and poison B labels, and is limited to one liter on passenger and 220 liters on cargo craft

IATA requires butyl bromide to bear the Red label, and limits it to one liter on passenger, 40 liters on cargo craft.

Allyl and propargyl bromides require flammable gas labels.

V. ENVIRONMENTAL CONTAMINATION

Presumably a large amount of methyl bromide is released into the air as normal operating procedure, as the products it is used to fumigate must be aerated before being consumed or processed further. Likewise soil sterilized by methyl bromide injection must be aired to minimize damage to seeds or seedlings.

A similar situation exists with the fumigating uses of ethylene dibromide. The latter has another route into the air by way of unburned gasoline from auto exhausts. No reports dealing with the extent of these emissions or effect on them of emission controls were found.

Leonard and Lider (1960) injected ethylene dibromide into soil and found that lateral diffusion was limited. This may mean that accidental spills onto soil in populated areas could be counteracted by prompt removal of the soil to a safer area.

The use, as indicated in Table 2, of certain bromohydrocarbons as flotation agents would release fumes into the air, and probably some liquid is trapped in any discarded materials.

VI. MONITORING AND ANALYSIS

Wade (1952) described a colorimetric analysis for bromide ion obtained from air samples containing bromohydrocarbons - not applicable if chloro- or bromochlorohydrocarbons are present. In a standard technique for sampling for this type of contamination, the air sample is drawn through a catalytic furnace into a bubbler containing alkaline hydrogen peroxide. This solution is transferred to a beaker and boiled down to 2-3 ml. After transfer to a calibrated test tube, and acidification with sulfuric acid, a fixed amount of aqueous $NaAuCl_4$ is added. The color developed is read against a reagent/treatment blank at 470 nm and compared with a calibration chart. No changes are required for the range 0.1-4.0 mg of bromide ion. Accuracy is less than silver nitrate titration, and better approximates the latter at the lower end of the range.

Lugg (1955) described a colorimetric method for determining a minimum of 50 mg/cu. m. (13 ppm) of methyl bromide in air. A 10-l sample of air is drawn through a one-l Winchester bottle. Add 15 ml of distilled pyridine containing 1% water; stopper and wet the bottle sides with the pyridine. Let stand seven hours, shake, invert, and let drain. To 10 ml of the solution in a 2.5 x 15-cm test tube, add 1/2 ml of 0.5N NaOH and heat for 15 minutes at 95°C. Cool five minutes in an ambient water bath. Add 1/2 ml of 2% aqueous India gum and read in a colorimeter within 10 minutes against water over the range 370-430 nm. There is a linear relationship between absorbance and amount of methyl bromide over the range 0-1.5 mg, but the minimum recommended is 0.1 mg. Ethylene dibromide interferes but not seriously, likewise ethyl chloride and ethylene dichloride; serious interference would come from methyl and ethyl iodides, and ethyl bromide.

Heseltine (1959) described a commercially available detector tube for methyl bromide which was suitable for monitoring fumigated enclosures.

Smith and Shigenaga (1961) described a technique for extraction of sterilants from soil. A 25 g soil sample, wetted with 20 ml of water, was shaken for 30 minutes with 2 ml of n-hexane for ethylene dibromide or o-xylene for propargyl bromide. A 5-50 µl aliquot of the extractant was then injected directly onto a gas chromatographic column. Recoveries ranged over 80-90%.

Alon et al (1962) analyzed for residual acetylene tetrabromide in ore from flotation processing, and in the hydrocarbon used to recover the bromohydrocarbon. Alcoholic KOH was used to remove the bromide as HBr. Oxidation to bromate could be followed by an iodometric finish when the sample contained 400 µg-10 mg of bromide in the presence of chloride, or by a colorimetric finish based on formation of tetrabromorosaniline, in the 0-20 µg bromide range. For the 10-200 mg bromide range, in the absence of chloride, an argentometric method was used, and for production control, the nephelometric method as silver bromide.

Dumas and Latimer (1962) analyzed atmospheric methyl bromide using $\leq$ 35-ml samples containing 1-100 mg/l of methyl bromide. After drawing the sample into an evacuated flask, 0.5 ml of 1N potassium hydroxide was added and heated at 60°C for 45 minutes. Excess alkali was neutralized with 1N nitric acid. Then the sample was titrated with a modified Fisher Coulomatic Titrator.

Dumas (1962) analyzed atmospheric ethylene dibromide by drawing a

sample into an evacuated flask, adding 1 ml of 0.5N sodium hydroxide, and refluxing for 15 minutes to remove one of the bromides. This was quantitized on a Fisher Coulomatic Titrator. Results were good for the 0.75-30 mg range.

Lindgren et al (1962) analyzed grain for residual combined bromide and methyl bromide after fumigation by neutron activation analysis. A 5-gm sample was irradiated for 30 minutes at a flux of 1.8×10^{12} neutrons/sq. cm. sec at a power level of 250 kW. The Br-82 0.77-Mev gamma ray intensity was measured after a 2-4 day decay period and compared with reference standards.

Woolfolk et al (1962) found that potassium p-phenylazophenoxide was a suitable derivatizing agent for alkyl halides, forming the alkyl ether. Refluxing the sample and the phenoxide in N,N-dimethylformamide for one hour and work up gave the following solid derivatives with m.p. in °C:

Methyl	52	Hexyl	58
Ethyl	72	Heptyl	69
Propyl	60	Octyl	73
Allyl	51	Decyl	64
Butyl	61	Hexadecyl	80
i-Butyl	63	Octadecyl	84
3-Methyl-butyl	37		

Ethylene dibromide gave a mixture of mono- and di- derivatives, mp 196-8°. Cyclohexyl bromide and tertiary halides did not react.

Takacs et al (1962) analyzed a mixture of methyl and ethyl bromides by gas liquid chromatography. Relative retention times were 1.0 for

methyl and 1.58 for ethyl under these conditions: column temperature, 52°C; carrier gas, H_2 at 75 ml/min and 1.0 kg/sq. cm. (1 atm); column, 5-mm i.d. by 3-m long; column packing, 20% β,β'-hydroxydipropionitrile on 30-60 mesh firebrick; detector, thermal conductivity cell at 52°C.

Castro (1964) gave a detailed description for the determination of methyl bromide in organic material which involved alkali/peroxide degradation and iodometric titration.

Bielorai and Alumot (1965) determined ethylene dibromide in organic materials by distilling 5-10 ml of benzene from a 2-l flask containing 100-300 g of sample and one liter of water, followed by gas liquid chromatography of the benzene. Results compared well with decomposition/titrimetry but were not as precise.

Berck (1965) used a 1/4-in. o.d. by 6-foot stainless steel column packed with 10% SE-30 on Diatoport S (60-80 mesh) to study conditions for separating methyl bromide, bromoform, ethyl bromide, ethylene dibromide, 1-,2-, and 1,3-dibromopropane, 1- and 2-bromobutane, 1- and 2-bromopentane.

Perry (1966) worked out the conditions for the use of an electron capture detector in the analysis of ethylene dibromide in gasoline. He used a 1/4-in. i.d. by 10-foot column packed with 5% Apiezon "L" and 0.5% polyethylene glycol 4000 on "Embacel" (100-120 mesh). The column and detector were maintained at 95°C. The carrier gas was nitrogen at 100 ml/min through the column, but only 15 ml/min through the detector to avoid overloading it. Elution time was six minutes. Reproducibility at the 30 ppm level was ± 3 ppm. The useful range was 1-50 ppm.

Chaudri and Hudson (1967) reported the following relative retention times for the separation of the isomeric butyl bromides on a glc column: t-butyl - 1.00, s-butyl - 1.94, i-butyl - 2.07, and n-butyl - 2.91. They used 1/16-in. o.d. by 4-m column packed with squalane (10%) on Chromosorb W, with nitrogen carrier gas at 15 ml/min and operating temperature of 20°C.

Getzendaner et al (1968) used a commercial X-ray fluorescence instrument to determine total bromide content of dry organic materials such as cereals and beans treated with methyl bromide. Calibration curves were obtained by analyzing material previously analyzed by chemical methods. At the 34 ppm level, precision was ± 10%.

Viel et al (1969) analyzed atmospheric methyl bromide by passing 20-40 l of air through twin absorbers (in ice) containing 20 ml each of freshly distilled diethylamine, then combining the contents of each bottle and evaporating the amine on a water bath. The residual hydrogen bromide was then dissolved in 5 ml of a buffer consisting of 1 part of 1N sodium hydroxide and 1.3 parts of 1N acetic acid (V/V). Then was added 1 ml of a solution consisting of 1 part of the buffer and 1/20 part of a phenolsulfophthalein solution (V/V). Then 1 ml of a 0.005 N aqueous chloramine T solution was rapidly added and let sit 30 seconds; 2 drops of 25% aqueous sodium thiosulfate were added to stop the reaction. The volume was adjusted to 10 ml and the optical density measured at 570 nm in a 1-cm cell. Amount of bromide was read from a curve prepared from 4-10 µg of bromide. The sensitivity limit was 4 µg, and the results were reproducible providing the HBr contacted no organic residues. Air contamination levels of 10-20 µg/l gave 90% recoveries, but 1-2 µg/l gave lower, and variable, results.

Malone (1970) described in detail an acid reflux procedure for extracting methyl bromide and ethylene dibromide from fumigated grain. Preliminary grinding was unnecessary for the EDB and detrimental for MB. The apparatus consisted of a one-l flask with N_2 inlet, a condenser with circulating 60°C (no higher) water, Teflon tube connection to a column of Chromosorb W (to remove traces of water), and a volumetric containing isooctane immersed in dry ice-acetone. The procedure was to add 100 g of sample to the swirled flask containing 530 ml water, 60 ml of 1N sulfuric acid, 10 ml of 20% phosphotungstic acid, and 1/2 ml of DC Antifoam FG-10 (spray antifoam was found unacceptable in connection with the subsequent glc). With a 25-30 ml/min flow of N_2, boil gently for two hours. Popping off of the tubing connection on top of the condenser meant that too much water had carried over, exceeded the capacity of the drying agent, and blocked the gas flow by freezing in the receiver. Rinse four 1-ml portions of isooctane through the tubing-drying agent using the N_2 pressure. Allow the isooctane solution to come to ambient, make up to volume, and inject a 5 µl sample into a 6-ft. x 4-mm i.d. column packed with 30% DC-200 on 80-100 mesh Gas Chrom Q. Operate the injection port at 150°C, the column at 70°C, and the electron capture detector at 200°C. Use a 60 ml/min flow of N_2 carrier. Unresolved problems of other volatile components in the grain held sensitivity to 3 ppm of methyl bromide and 0.3 ppm of ethylene dibromide.

Muthu et al (1971) used a bio-assay method for field determination of atmospheric methyl bromide (ethylene dibromide was tested and found unsuitable). They placed 30 adult red flour beetles, Tribolium

castaneum, in a U-tube and pumped the air sample through until all the beetles were "knocked-down", and noted the time required. Concentration was determined by dividing this time (in hours) into the predetermined C.T. product. The latter is found by averaging the values of knock-down time in hours multiplied by concentration in mg/l, using a range of concentrations. Their range for computing C.T. was 9-58 mg/l for methyl bromide. They tested chemically analyzed concentrations of about 2, 5, 10, and 52 mg/l and found quite acceptable values. Ethylene dibromide was unsuitable because it does not produce immediate kill.

Reilly (1971) showed in some preliminary modifications to a commercial methyl bromide leak detector that it was possible to make it useful at the TLV of 10 ppm by using propylene instead of propane as fuel and a ventilated copper tube reducer instead of the copper plate supplied.

Freedman et al (1973) demonstrated that standard charcoal filter respirator cartridges had a useful life of only one minute for methyl bromide and 17 minutes for ethyl bromide at a concentration of 50 and 5 times, respectively, the TLV's.

VII. CHEMICAL REACTIVITY

A. Environmental and use associated reactions

Ethylene dibromide's major use as a lead scavenger in leaded gasoline is to provide bromide atoms for the lead deposits, the lead bromide being volatile at engine operating temperatures. Presumably the bromohydrocarbon is decomposed at these temperatures, as Kirk-Othmer states that decomposition to vinyl bromide and HBr occurs at 340-370°C.

The chemical intermediate uses of the various bromohydrocarbons (RBr) rely upon their relatively weak carbon-bromine bonds. The bromine atom is readily displaced by O, N, S, and carbanions, these being said to be "alkylated". The RBr also readily react with finely divided Mg or Li to form RMgBr or RLi and LiBr; these so-called Grignard reagents are then used to attach the R group to carbon atoms which are far more electrically "positive" than the carbanions mentioned above.

Levine et al (1964) compared the ability of the various isomers of bromobutane to react with nitrogen dioxide and sunlight to form ozone. Table 3 is an adaptation of their table of results. They did not comment on the chemical fate of the bromobutanes. Butane itself

Table 3 –Ozone Generated by Sunlight Irradiation of 200 Pphm Butyl Halide + 100 Pphm NO_2

Butyl Compound	Induction Period (Min)	Maximum O_3 (pphm)
N-butane (control)	65	76
N-butane (control)	60	74
N-butyl bromide	35	27
N-butyl bromide	25	28
N-butyl bromide	35	26
Isobutyl bromide	25	34
Isobutyl bromide	30	32
Isobutyl bromide	30	37
Sec-butyl bromide	20	23
Sec-butyl bromide	45	17
Sec-butyl bromide	25	28
Tert-butyl bromide	90	12
Tert-butyl bromide	40	15
Tert-butyl bromide	60	16

generated much more ozone than any of its monobromo derivatives. The amount of ozone was corrected for normal ozone decay in a corresponding time period.

B. Aspects with biological implications

Clegg and Lewis (1953) treated barley, beans, groundnuts, maize, peas, rice, milled wheat, and whole wheat with methyl bromide, and did

not find any losses in nicotinic acid, riboflavin, or thiamine content. From in vitro treatment of solutions of nicotinic acid, nicotinamide, or thiamine with methyl bromide, they found apparent N-methylation of nicotinamide, < 4%, and free bromide in the solutions of the others.

Eaks and Sinclair (1955) found that ethylene dibromide acted as a ripener for avocados which had been fumigated with it.

Winteringham et al (1955) exposed samples of whole wheat flour of 12.5% moisture content (one batch of which had been prepared from wheat grown using S-35), and wheat gluten of 5 or 13% moisture content, to methyl bromide (C-14) for about 40 hours at 20°C. The samples were then aerated to remove free methyl bromide.

After a C-14 determination on a whole sample of flour, another sample was separated into fat, starch, gluten, and aqueous washes for individual C-14 measurements. The gluten samples were analyzed for N-, O-, and S-methylation. The results are in Table 4. The gluten (protein) fraction contained most of the C-14, the fat the least. Higher moisture content in the gluten itself decreased incorporation of methyl bromide. The C-14 activity in the aqueous washes was attributed to methanol via hydrolysis, and dimethylsulfide from thermal cracking of dimethylsulfonium salts (only the volatile components of the aqueous washes were determined). No S-35 was associated with N-methylated amino acids. A separate experiment involving fumigation of the S-35 flour indicated that dimethylsulfide was evolved naturally at ambient temperature, especially at higher humidity.

It was later shown (pp 261-8) that the principal (75%) reaction was with histidine; 1-N-methyl-, 3-N-methyl-, and 1,3-N-dimethylhistidines

Table 4.

Distribution of combined ^{14}C in wheat fractions following exposure to $^{14}CH_3Br$

Sample	A, Milled whole wheat		B, Milled whole wheat grown on sulphur-35		C, Wheat gluten		D, Wheat gluten	
Moisture content, %	12·5		12·5		5·0		13·0	
Fraction and method of ^{14}C recovery	p.p.m. of fraction	As % of total ^{14}C recovered in all fractions	p.p.m. of fraction	As % of total ^{14}C recovered in all fractions	p.p.m. of fraction	As % of total ^{14}C recovered in all fractions	p.p.m. of fraction	As % of total ^{14}C recovered in all fractions
(1) Whole wheat; total ^{14}C by wet oxidation	520	—	Spoilt	—				
(2) Fat; total ^{14}C by wet oxidation	164	0·6	38	0·7				
(3) Aqueous washings, volatile ^{14}C by distillation through combustion train (free $^{14}CH_3OH$)	16	3·0	28	18·4				
(4) Starch, total ^{14}C by wet oxidation	89	10·1	18	5·9				
(5) Gluten, total ^{14}C by wet oxidation	1687	86·3	427	75·0	2210	100·0	1351	100·0
		100·0		100·0				
		As % of total ^{14}C of gluten		As % of total ^{14}C of gluten		As % of total ^{14}C of gluten		As % of total ^{14}C of gluten
(5a) Gluten; $—O\cdot{}^{14}CH_3$, by difference, (5e − 5b − 5c)	171	10·1			290	13·2	101	7·5
(5b) Gluten, $—S\cdot{}^{14}CH_3$; by decomposition with NaOH followed by HCl	107	6·4			53	2·4	112	8·3
(5c) Gluten; $—S(^{14}CH_3)CH_3$; by decomposition with NaOH	555	32·9			548	24·8	362	26·8
(5d) Gluten; $—N\cdot{}^{14}CH_3$; by difference, (5 − 5e), cf with (5f) or (5g)	854	50·6	153	35·8	1316	59·6	776	57·4
		100·0				100·0		100·0
(5e) Gluten; $—O\cdot{}^{14}CH_3 + S\cdot{}^{14}CH_3 + S\cdot(^{14}CH_3)CH_3$, by HI hydrolysis	833	49·4	274	64·2	894	40·4	575	42·6
(5f) Gluten; $—N\cdot{}^{14}CH_3$, by decomposition in HI + NH_4I (Friedrich)	652				781		249	
(5g) Gluten; $—N\cdot{}^{14}CH_3$, by wet oxidation of HI hydrolysate used for (5e)					1046		586	

were isolated. It was concluded that loss of the semi-essential amino acid histidine by N-methylation was negligible from normal fumigation.

After consideration of literature relevant to consumption of methylated histidines and conjecture on human metabolic products (pp 269-73), it was decided that it was unlikely that methyl bromide fumigation of wheat would have toxic effects.

Siesto (1956) tested the effect of methyl bromide fumigation on the thiamine and riboflavin content of almond, nut, and pine seed meals. None of the treatments affected the riboflavin. Fumigation at

the 5 mg/l level at 18°C for seven days followed by seven days aeration had no effect on thiamine. The 1 g/l level for three days and seven days aeration reduced the thiamine to 50, 47, and 33% of unfumigated-but-aerated levels, respectively. Fumigation at the 1 g/l level for seven days reduced the thiamine to 25, 46, and 25% of untreated levels.

Bridges (1956) fumigated whole wheat for 48 hours with 29.5 mg/l of ethylene dibromide (Br-82); milled wheat, wheat gluten, and wheat starch were exposed at the 36 mg/l level. After airing, portions were heated at 180-200°C to simulate baking (one-half hour).

Pre-heating levels of water soluble bromide were low in comparison with methyl bromide, being concentrated in the gluten. After heating, these levels increased. The heating converted the ethylene dibromide adsorbed on the grain or fractions thereof into ethylene glycol and inorganic bromide. Some ether and/or ester formation occurred.

It was concluded that proper airing was very important for wheat fumigated with ethylene dibromide, and such being carried out, there should be little worry about residual fumigant or ethylene glycol baking byproduct.

Iwata and Sakurai (1956) tested methyl bromide on a variety of materials, measuring before and after bromide content: albumin 2.45 and 4.09%, casein 0.02 and 1.25%, gluten 0.06 and 0.73%, potato starch 0.08 and 0.09%, rice starch 0.02 and 0.05%, wheat starch 0.05 and 0.04%, defatted soybean meal 0.04 and 0.88% (the treatment of this meal decreased water-soluble nitrogen from 5.05 to 2.92 dry weight per cent). After treating coconut, linseed, and soybean oils followed by suction

evaporation of residual bromohydrocarbon, they found only a small change in the acid, iodine, and saponification numbers, viscosity, and bromide content.

Nachtomi (1972) recovered the microsome-supernatant fractions from the centrifugation of the homogenized livers of rats and chickens. Ethylene dibromide had no effect on the peroxidation ability of the lipids (catalyzed by NADPH) from rats, but inhibited the reaction of the lipids from chickens.

VIII. BIOLOGY

Winteringham and Barnes (1955) reviewed the literature on methyl bromide and ethylene dibromide, finding little that was pertinent to this section.

A. Metabolic Effects

1. absorption

Winteringham and Barnes (1955) in a review found that experiments with radioactive methyl bromide indicated it entered insects through their breathing apparatus and was also absorbed and decomposed on their skin. In another experiment no simple correlation was found between susceptibility to methyl bromide and respiratory activity of different stages of the confused flour beetle. Another species of this beetle genus was made more susceptible to methyl bromide by adding carbon dioxide to the atmosphere, a treatment known to increase the size of the openings of the air entrances in some insects.

It was known that skin contact of liquid methyl bromide in man caused blistering, but apparently no inquiry into possible systemic poisoning following such incidents had been done.

No studies had been conducted on ethylene dibromide.

2. excretion

Thomson et al (1958) gave rats s.c. injections of 1.25 g/kg of ethyl bromide. In the urine was found ethyl mercapturic acid, $C_2H_5SCH_2CH(NH\text{-}COCH_3)CO_2H$.

Bray and James (1958) reported that rabbits dosed with bromohydrocarbons excreted mercapturic acids in their urine. They identified only butyl mercapturic acid, from dosing with 1-bromobutane, but did find mercapturic acids from dosing with 2-bromobutane, bromocyclohexane, 1-bromo-heptane, -hexane, -octane, and -pentane. The longer the alkyl chain the lower the percentage of the dose eliminated as a mercapturic acid.

Grenby and Young (1959) isolated n-propylmercapturic acid from the urine of s.c. dosed guinea pigs, mice, rabbits, and rats.

Bray and James (1960) reported more results of their earlier (1958) study and follow up. Among these were the identification of pentyl and hexyl mercapturic acids from rabbit and rat urine. A second metabolite from rabbit urine after 1-bromobutane dosage seemed to be a peptide of S-butyl-L-cysteine and glycine. A third metabolite from this source seemed to be a more complex peptide containing S-butyl-L-cysteine, glutamic acid, glycine, and an unidentified sulfur compound. This third metabolite was the only one found in guinea pig urine after dosage with 1-bromobutane.

Grenby and Young (1960) published complete details of their 1959 report. They added that there was no evidence for the propyl mercapturic acid having been formed during the acid treatment of the urine

as a hydrolysis product of a mercapturic acid precursor, such as is seen with aromatic mercapturic acids.

They found that, in vitro, n-propylmercapturic acid was deacetylated by extracts of rat kidney or liver, but did not care to state positively that this may have occurred in vivo. Only 1/4-1% of the administered dose was isolated as pure mercapturic acid, but it was not thought that enough was lost in handling and purification to deny that this method of metabolism of the bromohydrocarbon is subordinate to still undiscovered pathways.

Thomson and Young (1960) reported finding in the urine of rats dosed with bromoethane (in addition to ethyl mercapturic acid) ethyl mercapturic acid-S-oxide, $C_2H_5SOCH_2CH(NHCOCH_3)CO_2H$.

James (1961) in still further elaboration of her 1958 study with Bray found that only negligible quantities of heptyl, and none at all of octyl mercapturic acid are present "as such" in rabbit urine after dosing with the appropriate bromohydrocarbon. Something is present which reacts the same with the nitroprusside detecting agent as free alkyl mercapturic acids do.

Bray et al (1964) dosed rabbits and rats with butyl, pentyl, hexyl, and heptyl bromides. They isolated from combined urines these purified mercapturic acids: butyl - 2% of dose in rabbits, 4% in rats; pentyl - 0.5% of dose in rabbits, 1.7% in rats; hexyl - 0.2% of dose in rats; heptyl - could not be crystallized (from rats), and could not be detected from rabbits (except after acid treatment and only in small amounts).

Octyl mercapturic acid could not be detected, directly or indirectly,

after dosage to either rabbits or rats. The same was found for butyl and hexyl mercapturic acids after dosage to guinea pigs. Table 5 contains their results including urinary bromide recovery from dosing with sodium bromide or bromoalkane.

Barnsley et al (1964) reported the metabolic pathways for ethyl bromide in rats diagrammed in Figure 1.

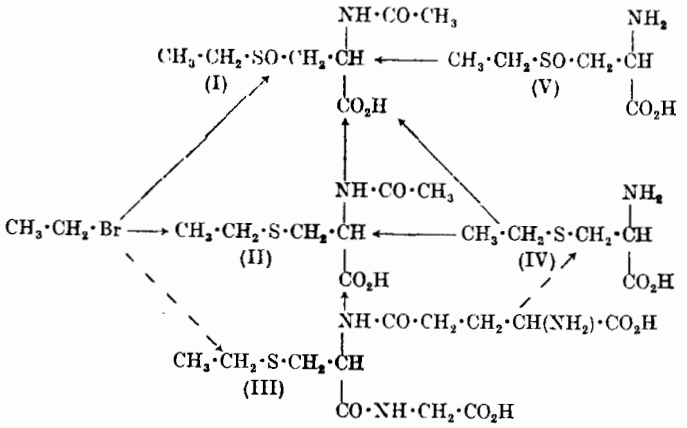

Figure 1. Biosynthesis of ethylmercapturic acid sulphoxide. Conversions demonstrated in the animal body are shown by solid arrows and possible conversions are indicated by broken arrows.

Barnsley (1964) reported finding in the urine of rats dosed with 1-bromopropane (in addition to 1-propyl mercapturic acid and 1-propyl mercapturic acid-S-oxide) 2-hydroxypropyl mercapturic acid. The yield was only 80 mg of the dicyclohexylammonium salt, from the combined urine of 32 rats given a total of 54 g bromopropanes.

Nachtomi et al (1966) gave rats stomach tube doses of ethylene dibromide, 100 mg/kg, as 2% solutions in soybean oil. The main urinary metabolite was β-hydroxyethyl mercapturic acid; in much smaller amount was found S-(β-hydroxyethyl)-L-cysteine.

Barnsley et al (1966) were unable to detect urinary sulfur-containing metabolites of 2-bromopropane given to rats by s.c. injection, except as traces and not consistently.

Table 5. Excretion of mercapturic acid and bromide by animals given bromoalkanes, or sodium bromide

Compound	Rabbit				Rat			Guinea Pig	
	Dose (m-moles/kg.)	Mercapturic acid	Apparent mercapturic acid	Bromide (96 hr.)	Bromide after 4 days	Dose (m-moles/kg.)	Mercapturic acid	Apparent mercapturic acid	Dose (m-moles/kg.)	Bromide (24 hr.)
1-Bromopropane	1·9	2·7 (1·3-5·2)[9]	-	-	-	2·3	5·1 (4·7-5·7)[6]	-	1·2	-
1-Bromobutane	1·7	4·7 (3·9-5·7)[8]	41 (27-54)[20]	16 (11-21)[6]	43 (41, 44)[2]	2·3	6·3 (4·0-8·1)[8]	54 (21-72)[16]	1·0	13 (9-16)[3]
1-Bromopentane	1·5	2·3 (1·6-3·7)[9]	21 (16-23)[3]	27 (18-40)[3]	50 (49-52)[3]	2·1	5·9 (2·8-9·0)[7]	35 (14-55)[6]	1·0	-
1-Bromohexane	1·3	0·9 (0·3-2·3)[7]	23 (21-25)[3]	29 (17-42)[4]	48 (45-53)[3]	2·0	3·1 (1·6-5·4)[11]	52 (23-69)[6]	0·9	-
1-Bromoheptane	1·1	N.D.[6]	16 (12-19)[3]	20 (11-22)[4]	66 (63-71)[3]	1·9	2·3 (1·0-3·1)[6]	70 (40-90)[6]	0·8	-
1-Bromo-octane	1·1	N.D.[6]	12 (0-21)[5]	19 (17-20)[3]	53 (47, 58)[2]	1·7	N.D.[6]	46 (24-59)[5]	0·7	-
Sodium bromide	1·7	-	-	12 (22, 25)[2]	48 (44-55)[3]	-	-	-	2·5	18 (16, 19)[2]

Amounts excreted are expressed as percentages of the dose, ranges are given in parentheses and numbers of experiments are indicated by superscript numbers. Unless the times are given, the results are for the amount excreted until the 24 hr. excretion returned to the normal level. N.D. indicates not detected; — indicates not examined. In the guinea pig, the excretion of true mercapturic acid was examined after administration of all the alkanes listed but none was detected. The value for apparent mercapturic acid was 18 (10-12)[4] from bromobutane (dose 1·0 m-mole/kg.).

James et al (1967) dosed rabbits with bromocyclopentane (A), -hexane (B), and -heptane (C). All gave methyl bromocycloalkyl triacetylglucosiduronates in the urine; in the case of B this metabolite was shown to be the 2-bromocyclohexyl isomer.

Also found in the urine from A-dosage were cyclopentyl mercapturic acid, 2-hydroxycyclopentyl mercapturic acid (also a metabolite from cyclopentene and epoxycyclopentane dosage), and another sulfur-containing material (also a metabolite from cyclopentene and epoxycyclopentane, and forming in vitro from cyclopentanone and N-acetylcysteine).

The major sulfur-containing urinary metabolite from B-dosage was an unknown material (also a metabolite of cyclohexene) which was not the 2-hydroxycyclohexyl mercapturic acid (a metabolite of epoxycyclohexane); in traces was found cyclohexylmercapturic acid.

Also found from C-dosage were small amounts of cycloheptyl mercapturic acid (a metabolite of cycloheptene), traces of 2-hydroxycycloheptyl mercapturic acid (also a metabolite of cycloheptene and epoxycycloheptane), and another sulfur-containing compound (also a metabolite of cycloheptene and epoxycycloheptane).

James et al (1968) gave female rabbits and rats stomach tube doses of aqueous suspensions of 1-bromobutane. The metabolites found in the urine are indicated in Figure 2. The rabbits excreted 8% of the dose as butyl mercapturic acid and 14% as hydroxybutyl mercapturic acids; the corresponding figures for rats were 7 and 22.

In a separate experiment bile duct-cannulated rats were given s.c. doses of 1-bromobutane. In the bile were found the metabolites S-butylglutathione, S-butyl-cysteine, and S-butylcysteinylglycine. Urine

collected over the same time period contained butyl and hydroxybutyl mercapturic acids.

A supernatant of rabbit liver homogenate produced S-butylglutathione and traces of S-butylcysteine when mixed with 1-bromobutane. Rabbit and rat liver slices produced 3-hydroxybutyl mercapturic acid from butyl mercapturic acid and from S-butylcysteine. Only rat liver slices produced 2-hydroxybutyl mercapturic acid. Rabbit liver slices produced 3-(butylthio)lactic acid, VIII in Figure 2, from S-butylcysteine.

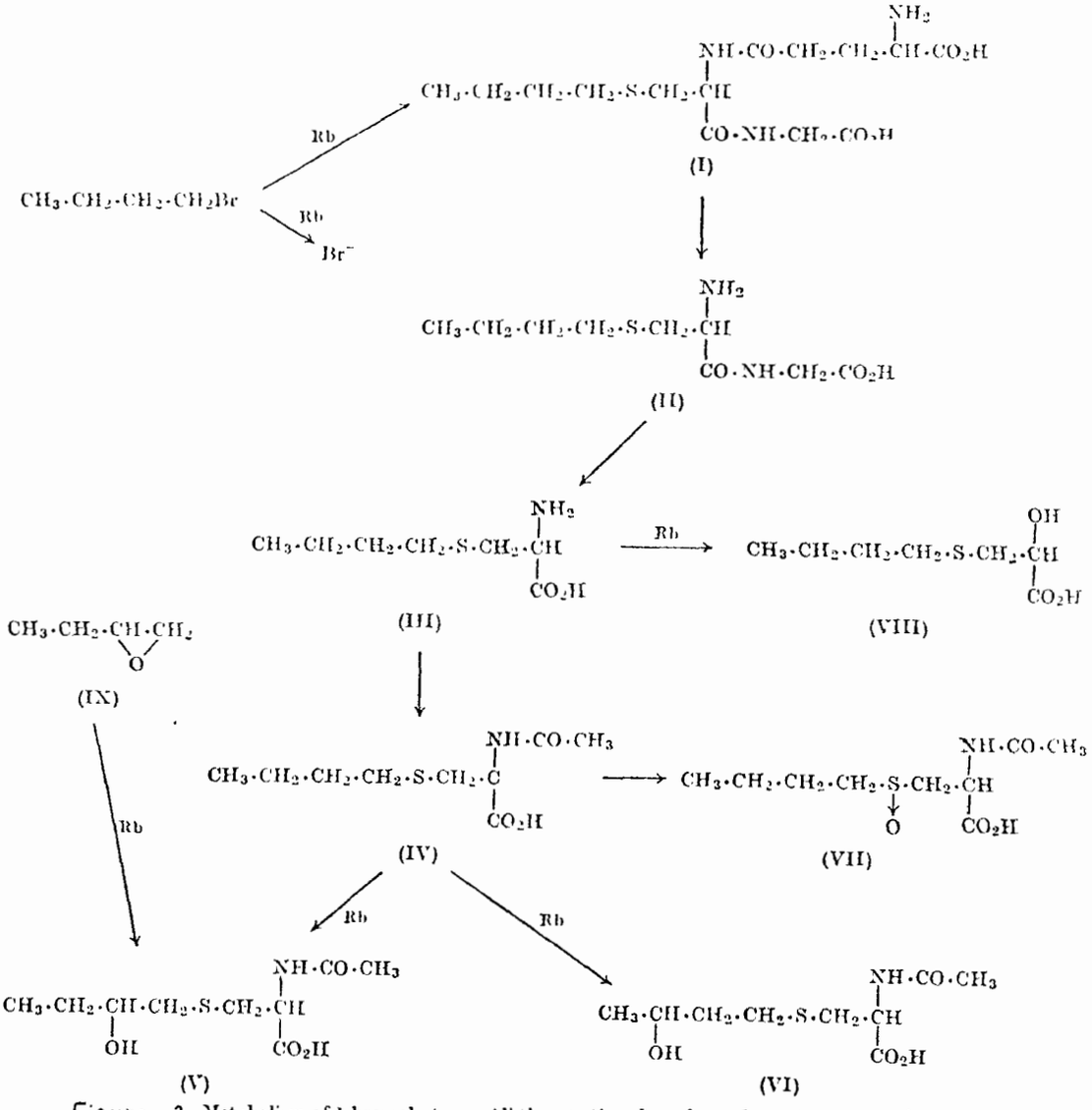

Figure 2. Metabolism of 1-bromobutane. All the reactions have been shown to occur in the rat, and those marked Rb in the rabbit.

Jones and Edwards (1968) gave rats oral doses of ethylene dibromide with both carbon atoms being C-14. They isolated 20% of the dose as CO_2. Metabolites found in the urine were S-(2-hydroxyethyl)cysteine, the S-oxide of the latter, hydroxyethyl mercapturic acid, and the S-oxide of the latter.

Jones and Howe (1968) added long-chain bromohydrocarbons to the yeast Torulopsis gropengiesseri in a glucose-containing medium. Methanolysis of the resultant glycolipids gave α,ω- alkane dioic acids (dimethyl esters). Starting bromohydrocarbon, product(s), and yield(s) were as follows: $CH_3(CH_2)_{14}$, $(CH_2)_{13}(CO_2-)_2$, trace

$CH_3(CH_2)_{15}$, $(CH_2)_{14}(CO_2-)_2$, 50%

$CH_3(CH_2)_{16}$, $(CH_2)_{15}(CO_2-)_2$, 45%

$CH_3(CH_2)_{17}$, $(CH_2)_{16}(CO_2-)_2$, 17%

& $(CH_2)_{14}(CO_2-)_2$, 29% (byproduct)

$CH_3(CH_2)_{17}$, $(CH_2)_{16}(CO_2-)_2$, 26%[a]

& $(CH_2)_{14}(CO_2-)_2$, 9%[b]

$CH_3(CH_2)_{19}$, $(CH_2)_{14}(CO_2-)_2$, 34%

$CH_3(CH_2)_{21}$, $(CH_2)_{14}(CO_2-)_2$, 36%

a - starting material and product both Δ^9 olefins

b - Δ^7 olefin byproduct of Δ^9 starting material

From $CH_3(CH_2)_{13}CH(CH_3)Br$ was obtained a low yield of $HO_2C(CH_2)_{13}CH(CH_3)Br$, but no glycolipids. From $CH_3(CH_2)_{13}C(CH_3)CH_2Br$ was obtained a 50% yield of $HO_2C(CH_2)_{13}C(CH_3)CO_2H$ (as the dimethyl ester).

Nachtomi (1970) gave rats stomach tube doses of ethylene dibromide. From the supernatant of the homogenized liver were isolated S,S'-ethylene bisglutathione, S-(2-hydroxyethyl)glutathione-S-oxide, and

S-(2-hydroxyethyl)glutathione; from the similarly treated kidneys were isolated S-(2-hydroxyethyl)glutathione and 2-hydroxyethyl mercapturic acid. When the bromohydrocarbon contained C-14 and the rats were sacrificed four hours after dosage, 6% of the C-14 was recovered (63% in the liver, 37% in the kidneys).

Kaye and Young (1970) injected rats with allyl bromide and found allyl mercapturic acid in the urine.

James and Needham (1970) found 4-carboxybutyl mercapturic acid in the urine of rabbits dosed with bromopentane and in the urine of rats dosed with 1-bromopentane or 1,5-dibromopentane.

James et al (1970) administered aqueous suspensions of bromocycloalkanes via stomach tube to female rabbits and examined their urine for metabolites. Sulfur-bearing metabolites are discussed in James et al (1971).

Bromocyclopentane gave bromocyclopentyl-tri-O-acetyl-D-glucosiduronic acid (isolated as the methyl ester). Bromocyclohexane and -heptane gave analogous compounds. Table 6 incorporates the results of urine analysis for total bromide, glucosiduronic acid, and ethereal sulfate.

TABLE 6. AMOUNT OF METABOLITES EXCRETED IN URINE BY RABBITS

Compound administered	Dose (m-mole/kg)	Total* bromide	Glucosiduronic acid	Ethereal sulphate
Bromocyclopentane	1·8	62 (54–73)$_6$	27 (16–38)$_6$	13 (12–16)$_3$
Bromocyclohexane	1·6	64 (58–76)$_6$	61 (35–84)$_3$	9 (8–10)$_2$
Bromocycloheptane	1·5	64 (56–71)$_3$	78 (69–85)$_6$	19 (17–22)$_3$

Results are means expressed as percentage of dose, with ranges in parentheses; the numbers of determinations are indicated by inferior figures

* This includes the bromide excreted plus organically bound bromine which is also estimated as bromide.

As measured by percentage of dose excreted as bromine, after 72

hours the order of metabolism was: cycloheptane = cyclohexane > cyclopentane > 1-pentane = 1-hexane > sodium bromide (all at same molar dose of Br). After 96 hours the only change was the near equivalence of the cyclopentane to the first two (the range for all compounds at this time being 47-64%).

Degradation of the glucosiduronic acid metabolites indicated that the rings had been attacked by oxygen at the carbon atom adjacent to that bearing the bromine atom.

James et al (1971) dosed rabbits and rats with bromocycloalkanes and examined the urine for metabolites. For the non-sulfur-bearing metabolites of the rabbits see James et al (1970). Tneir findings on mercapturic acid metabolites are in Table 7.

TABLE 7. [illegible] OF MERCAPTURIC ACID AND HYDROXYMERCAPTURIC ACIDS BY DOSED RABBITS AND RATS

[illegible]	Dose m-mole, kg	Rabbit				Rat			
		Mercapturic acid (%)				Mercapturic acid (%)			
		Alkyl	*cis*-2-OH Alkyl	*trans*-2-OH Alkyl	3-OH-Alkyl	Alkyl	*cis*-2-OH Alkyl	*trans* 2-OH Alkyl	3-OH-Alkyl
[illegible]	[illegible]	1·8 (1·6–2·0)^4	0·8 (0·6–0·9)^4	0·8 (0·6–0·9)^4	21·3 (19·6–22·2)^4	1·0 (0·8–1·2)^4	1·8 (1·4–2·2)^4	1·7 (1·3–2·1)^4	[illegible]
[illegible]	[illegible]	tr	tr	ND	10·3§ (9·9–11)^3	ND	ND	ND	11·1 (10·[illegible]–12·9)^3
[illegible]	[illegible]	1·2 (1·2–1·3)^3	ND	ND	5·5 (5·5)^3	0·6 (0–1·1)^3	ND	ND	4·2 (3·9–[illegible])^3

§- Includes trace amounts of other hydroxymercapturic acids. Amounts are expressed as percentages of the dose, ranges are given in parentheses and numbers of experiments are indicated by superscript numbers. ND indicates not detected; tr indicates trace amounts.

No S-oxides were detected. No other S-containing metabolites were detected by running the experiment on rats fed yeast labelled with S-35.

Grasse and James (1972) dosed rats with 1-bromopentane and

determined the metabolites with the assistance of [1-^{14}C]-1-bromopentane. Stomach tube aqueous suspension doses were given to adult female rats (only one rat received the radio-bromopentane). No S-oxides were found. Table 8 contains their findings of a positive nature. Metabolite 2 consisted of 2-, 3-, and 4-hydroxypentyl mercapturic acids. It was hypothesized that Metabolite 4 was a hydroxy derivative of 4-carboxybutyl mercapturic acid. The feces of the 24-hour period following dosage contained only 0.4% of the radioactivity present in the radio-bromopentane dose. No metabolites were detected in 72-hour feces after unlabelled bromopentane dosage.

Gas chromatography of the hydroxy metabolites as methyl esters gave the following relative peak areas: 4-hydroxy, 1; 3-hydroxy, 3; 2-hydroxy, 4; sulfate ester of the 3-hydroxy, 16; sulfate ester of the 2-hydroxy, 21. Until the detector sensitivity of these compounds has been determined, these figures should only be read as implying that the 2- and 3-hydroxypentyl mercapturic acids are excreted mostly as sulfate esters.

Table 8. Metabolites of 1-bromopentane excreted by the dosed rat.

	(*a*) 1-Bromopentane (% dose)	(*b*) [1-^{14}C]-1-Bromopentane (% dose of activity)*
Material determined		
Pentylmercapturic acid	7·0 (4·9–10·6)$_6$	4·5
Metabolite 2 (hydroxypentylmercapturic acid)	8·9 (6·8–12·0)$_6$	8·2
4-Carboxybutylmercapturic acid	2·4 (2·2–3·0)$_6$	2·4
Metabolite 4	n.d.¶	1·1
1-Bromopentane†	1·4 (1·3, 1·4)$_2$	n.d.
CO_2‡	n.d.	62·4
Bromide§	38 (35–42)$_2$	n.d.

Results are means expressed as percentages of dose with ranges in parentheses and numbers of animals as subscripts.

* Activity of dose was $1{\cdot}18 \times 10^6$ d.p.m.

† Excreted in expired air in 5 h after dose.

‡ 52% in 0–24 h and 10·4% in 24–120 h after dose.

§ Excreted 0–72 h after the dose when excretion was still incomplete. Rats dosed with an equivalent amount of NaBr excreted 31% (28–34)$_4$ of the bromide in 72 h.

¶ n.d. = not determined.

Sulfur-containing metabolites accounted for only 74% of those present in the urine after 24 hours - determined from the radiobromopentane experiment.

3. transport

Getzendaner (1965) gave chickens a layer ration containing an eight-fold range of bromide residue from methyl bromide fumigation. The bromide content of the whole eggs produced reached the same level, roughly, as that in the feed in 20-36 days. Figure 3 depicts the data graphically.

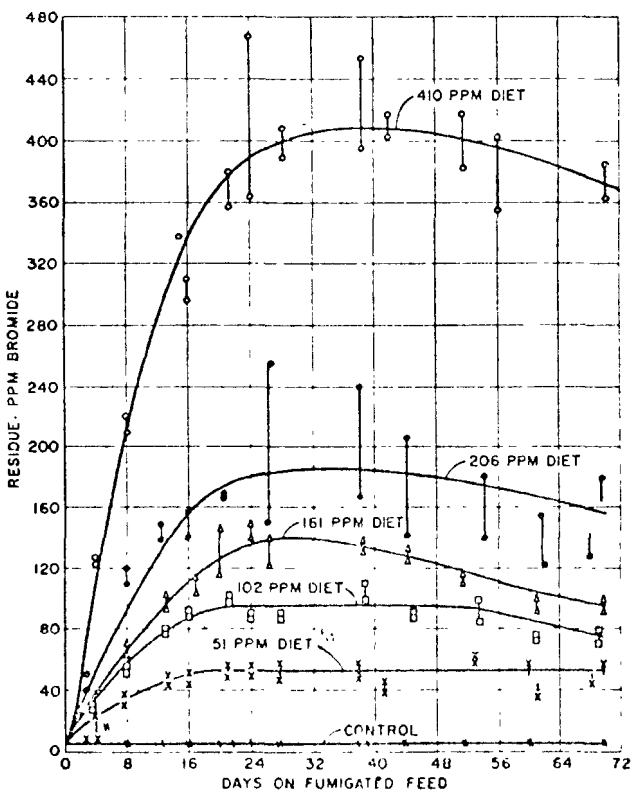

Figure 3. Bromide residues in eggs from chickens on fumigated diets

\ Control	△ 161-p.p.m. diet
× 51-p.p m. diet	◐ 206-p.p.m. diet
□ 102-p.p.m. diet	○ 410-p.p.m. diet

4. distribution

Getzendaner (1965) gave chickens a layer ration containing an eight-fold range of bromide residue from methyl bromide fumigation. His results on distribution of bromide in various portions of the hens and their eggs are presented in Figures 4 and 5, Tables 9, 10, 11, and 12. In 1965, the author commented, it was common practice to incorporate hydrolyzed chicken feathers as up to 2% of cattle feed and 5% in chicken feed. Since the average feather analysis for bromide residue was lower than that in the feed, there was no cause for concern about bioaccumulation.

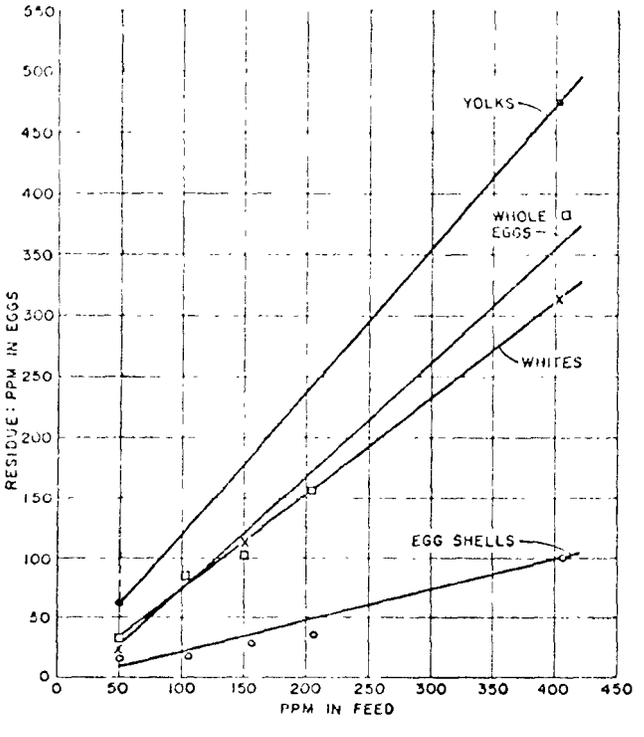

Figure 4. Residues of bromide in eggs vs. bromide content of feed

□ Whole eggs
○ Egg shells
● Yolks
× Whites

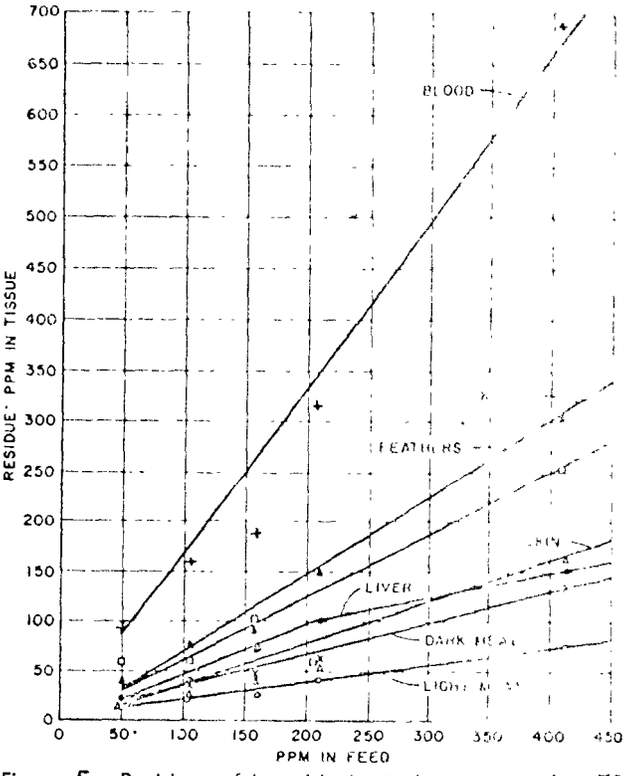

Figure 5. Residues of bromide in chicken tissues after 70 days on fumigated diets

○ Light meat
× Dark meat
● Liver
△ Skin
□ Feathers
▲ Kidney
+ Blood

Table 9. Bromide Residues Found in Egg White and Yolk

Days on Fumigated Feed	Br Found, P.P.M.			
	51 P.P.M in Feed		410 P.P.M in Feed	
	In white	In yolk	In white	In yolk
32	..	..	324	506
			280	480
33	32	57	293	450
			290	441
34	28	62	428	550
	26	71	..	
35	26	77	276	392
	24	56	..	..
36	34	76	..	.
Av.	30	67	315	473

Table 10. Rate of Bromide Accumulation after 70 Days on Feed

Tissue	Ratio: Av. P.P.M. in Tissue / P.P.M. in Feed	Ratio: Max. P.P.M. in Tissue / P.P.M. in Feed
Whole eggs (no shells)	1.0	1.3
Yolks	.2	1.5
Whites	0.8	1.1
Egg shells	0.3	0.5
Light meat	0.2	0.23
Dark meat	0.3	0.5
Skin	0.4	0.5
Liver	0.5	0.7
Feathers	0.6	1.5
Kidney	0.8	0.9
Blood	1.7	2.1

Table 11. Bromide Residues in Egg Shells

Bromide in Feed, P.P.M.	Days on Fumigated Feed		Bromide in Egg Shells, P.P.M.
Control	...		<13
			<13
			<13
		Av.	<13
51	46–66		<13
			36
			17
		Av.	19
102	41–54		13
			<13
			27
			21
		Av.	17
161	42–54		23
			25
			39
			29
			29
		Av.	29
206	42–56		44
			33
			42
			34
		Av.	38
410	42–70		103
			116
			96
			85
		Av.	100

Table 12. Bromide Residues Found in Chicken Tissues

Treatment and Tissue	Bromide Residues, P.P.M., Found after 28 Days, Bird 1	44 Days, Bird 2	56 Days, Bird 3	70 Days, Bird 4	Bird 5	Bird 6	Bird 7	Bird 8	Av.
Control									
Light meat	<5	<5	<5	<5	<5	<5	<5	<5	
Dark meat	<5	<5	<5	<5	<5	<5	<5	<5	
Liver	6	<5	<5	<5	<5	<5	<5	<5	
Kidneys	8	<5	<5	<5	<5	<5	<5	<5	
Skin	<5	<5	<5	<5	<5	<5	<5	<5	
Feathers	6	3	41	17	1	0.5	20	1.2	
Blood	..	...	...	...	<5	<5	<5	<5	
51 p.p.m. Br									
Light meat	11	9	10	12	15	14	14	15	14
Dark meat	13	13	14	13	18	18	19	18	17
Liver	33	28	39	21	28	21	26	30	25
Kidneys	45	29	47	36	44	39	44	35	40
Skin	30	28	26	12	12	13	18	14	14
Feathers	23	33	51	77	61	53	70	53	63
Blood	.	..	...	90	86	96	96	103	94
102 p.p.m. Br									
Light meat	16	23	19	23	24	23	28	23	24
Dark meat	28	28	26	29	39	33	42	33	35
Liver	40	61	53	36	39	50	39	32	37
Kidneys	65	92	71	68	74	81	66	72	72
Skin	50	36	26	21	25	29	41	22	28
Feathers	10	94	85	38	33	85	117	47	64
Blood	. .	..	...	146	157	171	162	144	156
161 p.p.m. Br									
Light meat	31	25	25	27	40	25	31	30	31
Dark meat	41	36	40	42	70	40	58	36	49
Liver	80	62	65	66	104	64	73	56	73
Kidneys	116	102	95	40	109	72	130	102	91
Skin	56	65	47	32	73	34	46	52	47
Feathers	15	86	83	61	34	82	158	174	102
Blood	...	...	...	222	232	189	241	219	181
206 p.p.m. Br									
Light meat	<5	38	33	44	42	46	44	42	43
Dark meat	8	49	32	49	64	64	71	70	64
Liver	18	108	74	62	121	65	112	144	101
Kidneys	26	140	113	163	122	134	152	172	149
Skin	19	95	62	80	47	47	53	66	59
Feathers	22	166	58	24	39	115	33	95	61
Blood	...	...	...	262	263	308	331	379	309
410 p.p.m. Br									
Light meat	66	63	60	95	88	108	. .	...	74
Dark meat	100	86	101	145	113	144	..		134
Liver	172	130	195	77	164	219	.	. .	153
Kidneys	344	176	288	308	292	304	. .	.	301
Skin	290	176	145	206	135	144	.		162
Feathers	34	61	312	133	515	102		..	250
Blood	..	...	...	702	703	670		.	692

Figures 4 & 5, Tables 9-12 reprinted with permission from J. Agri. Food Chem. 13:349-52 (1965), Copyright by the American Chemical Society.

B. Physiological Effects

Winteringham and Barnes (1955) reviewed the literature on methyl bromide and ethylene dibromide. The former produces nervous system damage of a nonpermanent nature, and interferes with enzyme function by reacting with SH-groups. The dibromide causes a non-narcotic type of unconsciousness, but no apparent nervous system damage. Minor hepatic and renal necrosis occurs.

Olomucki (1957) demonstrated that, in chickens, it was likely that ethylene dibromide caused the pituitary gland to decrease its production and/or release of follicle-stimulating hormone. This resulted in smaller than normal follicles, smaller than normal eggs, and, ultimately, cessation of egg production. An in vitro study demonstrated that the dibromide had no demonstrable effect on follicle-stimulating hormone.

Amir and Volcani (1965) administered 2 mg/kg/d of ethylene dibromide orally to male calves from four days after birth to 12 months of age, then changed the dosage to 4 mg/kg/every other day. They collected semen samples at 14-16 months of age, thereafter once a week for 8-10 months.

Health and growth were unaffected. Sperm density and motility were low. Malformations of the sperm included coiled tails, no tails, and degenerated pyriform heads. Recovery after discontinuation of dosage required 10-90 days. Onset of appearance of malformed sperm occurred two weeks either after first dosage to a 16-month old animal, or after resumed dosage in a previously dosed-from-birth animal after cessation of treatment.

Johnson (1965) gave an adult female rat a stomach tube dose of 1.16 mmole/kg of ethyl bromide. After two hours the hepatic glutathione level was 52% of normal. This compared with a literature value of 50% for a 1.6 mmole/kg dose of 1-butyl bromide.

Alumot (née Olomucki) and Mandel (1969) conducted additional experiments with chickens regarding ethylene dibromide, egg laying and size, and gonadotropic hormones. They decided that the conclusions drawn earlier (Olomucki, 1957) were incorrect, and that the dibromide's effect on chickens' egg laying was still unexplained.

Kazakova and Lis (1971) exposed mice four hours a day, five days a week for four months to an atmosphere containing 90 µg/l of 2-bromopentane. They observed an inhibition of neutrophilic phagocytosis, a suppressed development of local infectious inflammations, and an increased resistance to staphyloccal sepsis.

Alumot and Harduf (1971) fed laying hens feed containing 100 ppm ethylene dibromide. When the egg weight had dropped by one-third, the hens were given i.v. injections of radio-iodide labeled chick serum globulin (CGG) or albumin (CSA). As may be seen in Figure 6 the yolks of eggs laid after these injections incorporated only half as much of the protein fractions as non-dibromide fed controls. Similar results were found for incorporation of the labeled proteins into the vitelline membrane.

In another experiment CGG or CSA was given to control and dibromide-fed hens, who were sacrificed after 40 hours. The control hens had only half as many follicles as the treated hens, but the total weight was the same and uptake of radio-iodine was double that in the treated hens.

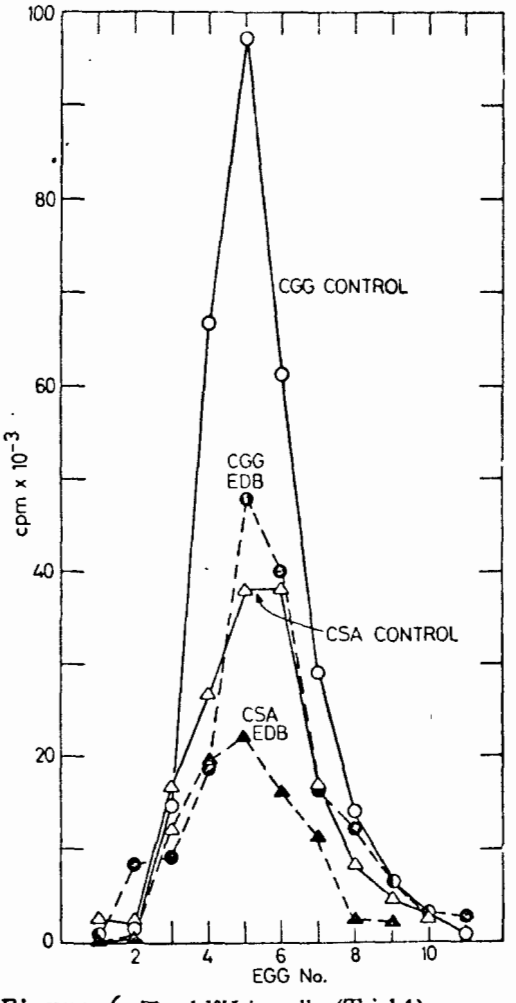

Figure 6. Total ^{125}I in yolks (Trial 1).
EDB - ethylene dibromide

James et al (1971) dosed rats with bromocycloalkanes and measured the hepatic glutathione after 1-4 hours. As seen in Table 13 there was a definite response to ring size.

Alumot (1972) reviewed the problems of ethylene dibromide reduction of egg size in chickens. The conclusion was that a reduction of protein uptake by the follicles resulted in slower growing follicles, hence, smaller eggs.

TABLE 13. EFFECT OF SOME MERCAPTURIC ACID PRECURSORS ON THE LEVEL OF TOTAL GLUTATHIONE IN RAT LIVER

Compound administered	Dose (m-moles/kg)	Total glutathione (mg/100 g liver) after:			
		0·5 hr	1·0 hr	2·0 hr	4·0 hr
Water only*		184 (176,191)[2]	187 187[1]	186 (186-187)[3]	186 (179-209)[2]
Bromocyclopentane	1·8	—	216 (201,230)[2]	202 (199,205)[2]	174 (169-179)[2]
	3·0	—	—	126 (103-157)[3]	
Bromocyclohexane	1·6	—	172 (155,189)[2]	194 (175,209)[3]	132 (131-134)[2]
	3·0	—	—	176 (173-178)[2]	
Bromocycloheptane	2·2	—	178 (171,185)[2]	145 (115,175)[2]	82 (64,99)[2]
	3·0	—	—	135 (138,132)[2]	

Dose suspended in water, administered at 0 hr to rats which had been fasted for 19 hr. Results are expressed as means with ranges in parentheses; the superior figure indicates the number of experiments. Control animals were given water by stomach tube.

IX. ENVIRONMENTAL EFFECTS

A. Persistence and/or Degradation

Coulon et al (1954) fumigated chestnuts with 17 or 118 g/cu.m. of methyl bromide. Analysis revealed bromide residues of 79-180 ppm, highest in the albumin, and dose rather than duration of treatment related. They recommended against this fumigation.

Olomucki and Bondi (1955) measured total and non-ether soluble bromide in samples of grain meals before and after extraction with ether. It was shown that the fat content served as a solvent for the ethylene dibromide used to fumigate the grain, but did not enhance the reactivity of the fumigant with the protein content.

Viel and Giban (1958) injected different types of soil with ethylene dibromide and left them undisturbed at or below 15°C for 8-9 weeks.

There was still dibromide present after this time, in a distribution gradient about the injection point. They recommended tillage after fumigation and before planting.

Lindgren et al (1962) determined that the total bromide residue in whole wheat (after fumigation with methyl bromide and aeration until no longer effective) increased rapidly with moisture content over the range 9-15% water, especially at higher fumigant concentrations. Over this same range higher residues were found the higher the temperature at which fumigation had been carried out (10-32°C range studied).

They compared bromide residues in various mill fractions obtained by milling after fumigation or fumigating after milling. In neither instance was there a correlation with fat content of any fraction. On a relative basis the residues in fumigated-milled fractions were: middlings - 1, flour - 1.1, whole - 1.6, shorts - 2.6, and bran - 2.9; the residues in milled-fumigated fractions were: whole - 1, middlings - 6.5, flour - 7.4, shorts - 7.9, and bran - 8.6. The only change in the order on going from post- to pre-milling was the whole grain dropping from the middle to the bottom. However, residues in the fractions were 2-5 times higher when pre-milled, even at slightly lower moisture content.

Sinclair et al (1962) fumigated whole and milled wheat with ethylene dibromide. After 10 days standing at 21°C, about 97-98% of the added dibromide could be recovered unchanged from either type of sample. The relative total bromine residues in milled-fumigated fractions were: whole - 1, shorts - 2.6, middlings - 3.4, bran - 3.6, flour - 3.6+. The order of ionized bromine residues was: whole - 1, shorts - 1.8, flour - 2.4, bran - 2.8, and middlings - 3.3.

Sinclair et al (1964) showed that corn and wheat (9% water) absorbed

about the same amount of ethylene dibromide when fumigated at 10-20°C, but corn absorbed more at a higher temperature. Studying the effect on fumigant retention of such variables as moisture content, fumigation temperature, and post-fumigation storage temperature, the authors found an increased retention from increases, increases, and decreases, respectively, for wheat, and increases in all three for corn.

Recovery of ethylene dibromide added to ground corn did not drop below 90% after 10 days sitting at 20°C.

Getzendaner et al (1968) fumigated commercial foodstuffs, flavorings, and baking products with methyl bromide using conditions designed to simulate current practice but slanted to yield maximal bromide residues. Some of their tested goods may already have been treated with bromide fumigants. Their results are in Table 14. The last column on the right was intended to be used as an aid in predicting residue accumulations for repetitive fumigations. The 1966 Federal Register tolerance levels for bromide residues in processed foods were partially based on the results presented herein.

Castro and Belser (1968) incubated soil, nutrients, and ethylene dibromide-1,2-C-14. Within eight weeks nearly all of the fumigant had been converted to ethylene and bromide ions. When meso-2,3 dibromobutane was used, trans- or cis-2-butene were produced, respectively, indicative of stereospecific trans elimination of the Br's. Quantitative analysis of this compound could not be run because of hydrolysis observed in the sterile control cultures; however, no olefinic or gaseous products were seen in the controls.

Brown and Jenkinson (1971) fumigated soil with methyl bromide at

Table 14. Bromide Residues in Food Commodities

Food	H_2O, %	°F.	Hours	Rate, Lb./M.	Bromide Residues, P.P.M. Untreated	Treated (net)[a]	Specific[b]
Candy and Confections							
Candy bar[c]	1.3	80	24	1	1, 1	8, 5	6
Brand 1		80	24	3		26, 18	7 3
Chocolate bar	1.6	60	12	3.75	0, 0	25	6 7
Brand 2		80	12	2.5		26	10
Chocolate bar	1.3	80	24	1	2, 2	6, 7	6.5
Brand 3		80	24	3		18, 17	5.8
Candy bar[c]	1.2	80	24	1	1, 4	8, 7	7 5
Brand 4		80	24	3		28, 13	6 8
Candy bar[c]	1.2	80	24	1	3, 2	10, 8	9
Brand 5		80	24	3		31, 31	10
Fudge	0.6	80	24	1	2, 2	3, 3	3
Brand 6		80	24	3		11, 4	2 5
Chewing gum	2.1	80	24	1	0, 1	4, 3	3.5
		80	24	3		9, 10	3.2
Marshmallows, large	0.7	60	12	3.75	0, 0	6	1 6
		80	12	2.5		7	2.8
Marshmallows, miniature	1.4	80	24	1	0, 0	0, 0	0
		80	24	1.5		0, 0	0
Mints[c]	0.8	80	24	1	1, 2	5, 1	3
		80	24	3		6, 12	3
Peanuts[c]	1.8	80	24	1	4, 13	27, 5	16
		80	24	3		59, 55	19
Sugar, cane[d]	0.1	80	24	1	7, 7	0, 0	0
		80	24	1.5		0, 0	0
Cereal Products							
Corn flakes	3.9	80	24	1	5, 6	0, 0	0
Brand A		80	24	1.5		0, 2	0.7
Corn flakes	4.7	80	24	1	5, 6	0, 2	1
Brand B		80	24	1.5		0, 2	0 7
Hominy grits	8.2	80	24	1	4, 3	6, 11	8 5
		80	24	1.5		10, 11	7 0
Oat cereal	4.3	80	24	1	0, 0	8, 7	7.5
		80	24	1.5		15, 14	9.7
Puffed rice	4.1	80	24	1	0, 0	7, 9	8
Brand A		80	24	1.5		6, 7	4.3
Puffed rice	5.8	80	24	1	0, 0	9, 9	9
Brand C		80	24	1.5		10, 14	8
Cream of wheat	11.1	80	24	1	12, 25	17, 17	17
		80	24	1.5		34, 44	26
Puffed wheat	4 9	80	24	1	0, 0	6, 8	7
		80	24	1.5		9, 11	6 7
Shredded wheat	6 9	80	24	1	0, 10	5, 8	6.5
		80	24	1 5		11, 4	10
Flaked cereal	7.9	60	12	3.75	8, 5	14	3 7
Brand D		80	12	2.5		7	2 8
Baking powder	11.6	60	12	3.75	0, 0	0	0
		80	12	2.5		0	0
Noodles, egg	6.7	80	24	1	0, 0	15, 16	16
		80	24	1.5		23, 20	14
Starch, corn	10.6	80	24	1	3, 3	3, 2	2.5
		80	24	1.5		5, 5	3.3
Macaroni	9 6	60	12	3 75	0, 0	12	3.2
		80	12	2.5		12	4.8
Dog food, dry	8.9	60	12	3.75	8, 11	98	26
		80	12	2.5		81	32
Cattle feed, mixed	10.3	60	12	3.75	8, 0	68	18
		80	12	2.5		81	32
Cornmeal	11.9	60	12	3.75	0, 0	68	18
		80	12	2 5		68	27
Flour, rice	8.5	80	24	1	106, 107	0, 0	0
		80	24	1.5		13, 53	22
Flour, soy	6 0	70	42.5	1	0	49	49
Flour, tapioca		70	42 5	1	0	0	0
Flour, white wheat							
Brand F[e]	8.5	80	24	1	14, 16	41, 41	41
		80	24	1.5		53, 61	38
Brand 2[e]	11.3	80	24	1	12, 13	21, 18	20
		80	24	1 5		26, 30	19

Table 14. (Continued)

Food	H_2O, %	°F.	Hours	Rate, Lb./M.	Bromide Residues, P.P.M. Untreated	Treated (net)[a]	Sp[illegible]
Brand 2A[c,d,h]	11.3	80	24	1	15, 16	23, 24	24
		80	24	1.5		32, 45	2
Brand 3[e,g,h]	11.1	80	24	1	10, 7	28, 29	2[illegible]
		80	24	1.5		32, 41	24
Brand 4[e]	8.8	80	24	1	6, 0	21, 23	22
		80	24	1.5		40, 51	30
Flour, whole wheat							
Brand 5[e]	10.5	80	24	1	14, 13	28, 32	36
		80	24	1.5		46, 44	30
Brand 6	8.2	80	24	1	7, 5	36, 41	3
		80	24	1.5		60, 52	37
Brand 7	11.4	60	12	3.75	0, 0	79	22
		80	12	2.5		92	36
Cake mix							
Brand 8	4.3	60	12	3.75	0, 0	24	6
		80	12	2.5		19	7
Brand 9	3.8	80	24	1	4, 5	3, 1	2
		80	24	1.5		7, 7	4
Brand 10	4.8	80	24	1	4, 4	3, 3	3
		80	24	1.5		6, 1	2
Brand 11	4.1	80	24	1	4, 5	5, 7	6
		80	24	1.5		10, 6	
Pancake mix							
Brand 12	8.4	80	24	1	8, 8	7, 7	7
		80	24	1.5		10, 10	9
Brand 13	8.5	80	24	1	31, 32	0, 0	0
		80	24	1.5		0, 0	0
Pie crust mix							
Brand 14	6.4	80	24	1	14, 13	6, 7	6
		80	24	1.5		13, 16	9
Brand 15	6.2	80	24	1	5, 4	4, 2	3
		80	24	1.5		5, 8	4
Brand 16	7.2	80	24	1	22, 27	10, 16	
		80	24	1.5		22, 28	1
Animal Products, Fats							
Cheese, cheddar	5.1	80	24	1	5, 4	8, 16	12
		80	24	3		31	10
Cheese, cottage, creamed	50	80	24	1	1, 2	7, 10	8
		80	24	3		30, 32	15
Cheese, pimenting	13	80	24	1	1, 2	8, 9	8
		80	24	3		37, 23	19
Cheese, parmesan, grated		80	24	1	7, 8	85, 75	80
		80	24	3		252, 190	7[illegible]
Beef, roast, chuck	57	80	24	1	1, 2	24, 13	19
		80	24	3		37, 65	17
Beef, roast, loaf	36	80	24	1	4, 4	11, 16	14
		80	24	3		58, 30	15
Frankfurters, skinless	35	80	24	1	4, 5	31, 27	29
		80	24	3		85, 73	27
Pork, shoulder, smoked	37	80	24	1	3, 3	6, 8	7
		80	24	3		47, 21	12
Pork, steak	37	80	24	1	4, 2	28, 22	25
		80	24	3		50, 53	17
Bacon, sliced	11.7	80	24	1	6, 7	22, 29	25
		80	24	1.5		58, 37	32
Eggs, powdered		80	24	1	36, 36	83, 125	108
		80	24	3		354, 338	11
Gelatin, unflavored		80	24	1	18, 20	11, 13	13
		80	24	3		60, 53	1
Gelatin, flavored	1.8	60	12	3.75	0, 0	0	1
		80	12	2.5		0	0
Milk, malted		80	24	1	9, 10	7, 3	5
		80	24	3		0, 5	1
Dry		80	24	1	10, 8	6, 7	
Skimmed		80	24	3		5, 5	1
Milk, dry		80	24	1	34, 37	2, 1	1
Whole		80	24	3		13, 9	5
Pork sausage, link	19.5	80	24	1	3, 6	17, 11	15
		80	24	3		35, 50	13

Table 14. (Continued)

Food	H_2O, %	°F.	Hours	Rate, Lb./M.	Bromide Residues, P.P.M. Untreated	Treated (net)[a]	Specific
Veal loaf	40	80	24	1	6, 6	8, 8	8
		80	24	3		27, 25	8 7
Butter	4.7	80	24	1	1, 1	6, 4	5
		80	24	3		11, 11	3 7
Oleomargarine	4.4	80	24	1	3, 3	1, 0	0.5
		80	24	3		3, 7	1.7
Shortening	0.7	80	24	1	1, 1	1, 1	1
		80	24	3		2, 8	1.7
Herbs, Spices, Beverages, Misc.							
Cocoa	6.0	60	12	3.75	0, 0	47	13
		80	12	2 5		33	13
Coffee, ground	5 7	60	12	3.75	0, 0	25	6.7
Brand A		80	12	2 5		29	12
Coffee, ground	2.9	80	24	1.0	1, 1	11, 12	12
Brand B		80	24	1.5		10, 10	6.7
Coffee beans	2.7	80	24	1	0, 0	4, 4	4
Roasted		80	24	1.5		5, 5	3.3
Tea, green	5.3	80	24	1	6, 6	0, 0	0
		80	24	1.5		5, 7	4
Tea, orange pekoe	6.1	80	24	1	7, 8	0, 0	0
		80	24	1.5		1, 2	1
Allspice, ground	8.6	80	24	1	4, 4	15, 25	20
		80	24	1.5		26, 23	17
Cinnamon, ground	9.0	80	24	1	4, 3	12, 0	6
		80	24	1.5		19, 16	12
Ginger, ground	10.4	80	24	1	73, 71	8, 8	8
		80	24	1.5		6, 11	6
Nutmeg, ground	7.9	80	24	1			
		80	24	1.5	25, 24	7, 4	5.5
						16, 11	9 1
Pepper, red, ground	7.3	80	24	1	28, 28	9, 9	9
		80	24	1.5		6, 5	3 7
Yeast, dry	7.5	80	24	1	1, 1	0, 0	0
		80	24	1.5		0, 0	0

[a] Net = residue in treated − average residue in untreated sample. [b] Specific residue = $\frac{\text{av. p p m. increase from fumigation}}{\text{rate of fumigation (lb. M.)}}$. [c] Chocolate-covered. [d] Extra fine granulated. [e] Bleached. [f] Bromated. [g] Fancy patent. [h] Enriched. [i] Pastry flour. [j] Fumigated in commercial 6-oz. package.

98 kg/ha. Crops of wheat grown on the soil the same year and two succeeding years contained 0.42, 0.25, and 0.09% bromide in the above ground portions - first year plants which suffered scorching damage contained as much as 0.61%. Otherwise similar soils containing 0.93% or 2.81% organic carbon retained 25 and 63 ppm bromide, respectively.

Dumas (1973) fumigated fresh fruits and walnuts with methyl bromide and/or ethylene dibromide and determined the residues. Results are in Tables 15-19. In general lower fumigation temperatures resulted in lower bromide (Br^-), but higher ethylene dibromide, residues.

Table 15. Bromide Residues Found in Pulp and Skin of Fruits Fumigated with Methyl Bromide at Various Temperatures. Ex, Normal Atmospheric Pressure (760 mm) for 2 hr

		Residues as bromide, ppm				
		Peach		Cherry		
Temperature, °C	Dosage methyl bromide, mg/l	California	Ontario	California (Bing)	California (Schmidt)	Pl
25	0 (control)	0.3	0.2	4.1	3 0	
25	16	3.5	4.9	11.4	6.7	1
25	24	4.5				
25	32	5.5				
21	24	4.0	4.2	11.0	6.1	
21	32	4.6				
21	40	5.3				
15	32	3.5	3.2	8.2	5.9	
15	40	4.0				
15	48	4.3				
10	40	2.7	2.3	7.2	4.6	
10	48	3.2	3.9	8.2		3
10	64	3.8				
4	48	1.9	2.4	4.2	3.5	
4	64	2.7	4.7	5.3	4.7	
4	80	3.5				

Table 16. Distribution of Residue in Peaches and Plums (ppm) Fumigated with Methyl Bromide at Different Dosages and Temperatures for 2 hr

		Dosage methyl bromide, mg/l		
		0	16	64
		Temperature, °C		
	Fruit part	25	25	4
Peaches	Skin	4.3	10 4	3.4
Peaches	Pulp, outside half	2.0	1.8	1.9
Peaches	Pulp, inside half	2.0	5.2	2.3
Peaches	Pit wall	1.3	4.1	1.1
Peaches	Seed	15.0	47.0	15.7
Plums	Skin	4.7	7.1	8.6
Plums	Pulp	1.0	1 9	1.8
Plums	Pit	2.2	5.2	6.2

Table 17. Residues of Ethylene Dibromide and Inorganic Bromide in Apples[a] after Fumigation with Ethylene Dibromide and 24 mg/l. for 4 hr at 13°

Concentration, mg/l.	Time after fumigation, days	Ethylene dibromide residue, ppm	
12	1	36	
12	2	14	
12	3	4.5	
12	6	1.2	
12	12		
24	1	75	
24	2	40	
24	3	13	
24	6	1.6	
Control nonfumigated apples		0	

[a] Apples (Delicious variety) held in cold storage 10 months and [illegible] at 13° after treatment. [b] This includes some bromide resulting from the ethylene dibromide

Table 18. Residue of Ethylene Dibromide and Inorganic Bromide in Newly Harvested MacIntosh Apples after Fumigation with Ethylene Dibromide 12 mg/l. for 4 hr at 13°

Concentration, mg/l.	Time after fumigation, days	Storage temperature, °C	Ethylene dibromide, ppm	Inorganic[a] bromide residue, ppm
12	1	13	23	1.7
12	2	13	3.6	2.2
12	4	13	1.2	
12	6	13	0.17	2.4
12	9	13	0.14	
12	12	13	0.23	1.9
12	2	25	0.2	
12	3	25	0.15	
12	5	25	0	
Control nonfumigated apples			0	0.8

[a] This includes some bromide resulting from the ethylene dibromide

Table 19. Inorganic Bromide Residue in Shelled and "In-Shell" Walnuts after Exposure to Methyl Bromide at Various Concentrations for 24 hr

	Fumigation temperature, °C	CH_3Br dosage, mg/l	Inorganic bromide residue, ppm
Without shell	25	0	2
	25	16	90
	21	24	81
	15	32	74
	10	40	67
	5	58	110
	2	64	45
	−1	64	6
	−4	64	72
In shell	25	16	22
	21	24	14
	15	32	24
	10	4	14
	5	58	17

B. Environmental Transport

No specific information was found. When used in above ground applications, methyl bromide and ethylene dibromide evaporate into the general atmosphere. When used in below ground applications, methyl bromide has to be "covered" to prevent rapid evaporation into the air, and ethylene dibromide seems to stay very close to its point of insertion.

C. Bioaccumulation

Martin et al (1956) grew vegetables and citrus seedlings in soil treated with ethylene dibromide. In either a sandy loam or a silty clay loam the citrus tops accumulated only about 0.40% of Br over the range 1-12 ml ethylene dibromide per 3 gallons of soil; at the 0.5-0.8 ml/3 gal of soil level, accumulation was about 0.17%. Lima bean and carrot tops accumulated more Br than the citrus seedlings, 1.35 and 0.60%, respectively, at 8 ml/3 gal. Carrot tubers acquired only 0.10% at 8 ml/3 gal.

Munsey et al (1957, pp. 201-2) added 13 ppm of ethylene dibromide to commercial bakers' flour and 20 ppm to rolled oats. Bread prepared from the flour was free of unchanged fumigant. Boiling the oats in water for one minute left 12 ppm of the fumigant in the oats.

Young et al (1959) allowed cows to feed only on peanut vines grown on ethylene dibromide treated soil. After 28 days of increasing Br content in the milk, these levels ranged 14-61 ppm from vines containing 48-314 ppm, respectively. The bromide levels were rising at a rapid rate when the experiment was terminated.

Muns et al (1960) grew a variety of vegetables on earth treated with ethylene dibromide at 4.67 g/m^2. Lima bean straw contained 18, 76, and

28 ppm Br when harvested 20, 19, and 16 weeks after soil treatment. Corresponding sets of figures (ppm, weeks) for other crops were:

onion - 9.1, 16

beet - 10.4, 10

turnip - 10.8, 8

spinach - 11.1, 8

lettuce - 14.9, 14

sugar beet tops - 18.3, 18

roots - 30.4, 16

shelled blackeye bean - — , 14 (none detected)

Lynn et al (1963) fed cows a ration consisting in part of methyl bromide fumigated oats and corn. There was a direct correlation between the Br content of blood and milk. Dietary levels of 10, 19, and 43 ppm Br produced milk levels of 4-12, 7-12, and 10-20 ppm, respectively. The Br levels plateaued at 4-5 weeks at the low and middle Br-in-feed levels, and at 2-3 weeks at the high level. Milk contained a higher percentage of Br taken in as a contaminant in the feed than as a NaBr dietary supplement.

Getzendaner (1965) fed hens feed containing 50-410 ppm of Br from methyl bromide fumigation. The following portions of the hens and eggs were examined for Br content after 70 days (figures are average Br/Br in feed, max. Br/Br in feed):

light meat - 0.2, 0.23

dark meat - 0.3, 0.5

egg shells - 0.3, 0.5

skin - 0.4, 0.5

liver - 0.5, 0.7

feathers - 0.6, 1.5

kidney - 0.8, 0.9

egg whites - 0.8, 1.1

egg yolks - 1.2, 1.5

blood - 1.7, 2.1

Thus, except in the blood and egg yolks, the hen did not accumulate Br over the amount it was taking in.

Wilson and Norris (1966) applied ethylene bromide to soil at 11 ml/m^2 annually for nine years. Table 20 contains the Br content of the soil and various crops grown on it in the last year. The figure for onions is complicated by the poor growth of onions in this treated soil. There is no apparent correlation between the accumulation in the various root crops.

Laue et al (1969) fed cows, calves, and piglets for 90 days on a diet which had been methyl bromide-fumigated. A plateau was reached in the blood and organs for a particular Br intake. The Br content of milk and flesh was not hazardous for human consumption.

X. TOXICITY

A. Human-Occupational experience, Other

Prain and Smith (1952) discussed an occurrence in 1947 in which six of eight boys died after exposure to methyl bromide from a fire extinguisher in a confined area. Pre-death symptoms included convulsions, epileptiform fits, and depressed reflexes. Massive pulmonary edema was evident, and anuria became obvious within one day. The first urine passed by the two survivors contained considerable amounts of albumin and many granular

TABLE 20.

Total bromides in p p m. in various crops and the muck soil in which they were growing after 9 years of treatment with the same fumigants. 1965 data.

Vegetable	Sampling date	EDB
Radish	7/20	
Roots		378
Tops		665
Soil		16.5
Beet	8/2	
Roots		216
Tops		469
Soil		16.5
Lettuce	9/28	
Leaves		330
Soil		27
Carrot	9/29	
Roots		45
Soil		27
Potato	9/29	
Tubers		66
Soil		27
Onion	9/29	
Bulbs		12
Soil		27
Celery	8/25	
Stalks		402
Soil		18.5
Spinach	8/10	
Leaves		102
Soil		18.5

casts; neither of the survivors showed pulmonary edema signs.

The authors conjectured that a toxic dose of methyl bromide caused damage to the periphery of the respiratory system and to the renal tubular epithelium, and also caused cerebral upset.

Gallais et al (1952) discussed another case of methyl bromide leaking from extinguishers in which one of three adults hospitalized later died. All suffered from cerebral disturbances, dysarthria (speech difficulty), bilateral mydriasis (dilated pupils), and swallowing problems. The autopsy revealed extensive necrosis of the greater curvature of the stomach,

hemorrhagic gastroduodenitis, brain congestion, and massive hepatic fatty degeneration.

Kubota (1955) reported that human fatalities resulted from air concentrations of methyl bromide $\geq$ 600 ppm, but 100-150 ppm was harmless. The blood of fatal cases had 211 mg/l of Br, with survivors showing only 50 mg/l. Skin in contact with 8000 ppm developed pustules.

Winteringham and Barnes (1955) reviewed the symptomology of methyl bromide poisoning. There is a latent period following exposure even to an eventually toxic dose. Headaches, dizziness, nausea, vomiting, weakness, mental confusion, restlessness, mania, and finally tremors and convulsions preceed death, usually from pulmonary edema.

Allen (1956) reported that there were no reported cases of fatalities or even dermatitis from exposure to ethylene dibromide. Overexposure to vapors produced irritation to the eyes, nose, and throat, headache, giddiness, nausea; chronic overexposure damaged liver and kidneys. No standard for maximum air concentration had been set, but the range 2-25 ppm was under consideration.

Turner (1958) reviewed the toxicology of di- and tetrabromomethanes, recommending industrial exposures of < 25 ppm for CH_2Br_2 and < 1 ppm for CBr_4/8-hr.

Fiorentini and Mosinger (1958) described two fatalities from exposure to 3 mg/l of methyl bromide. Inflammation and degenerative changes in the cerebellum, cortex, pallido-striatum, and thalamus accompanied cerebral edema. Lower down, the heart muscle, kidneys, liver, and lungs exhibited degenerative-inflammatory changes, hemorrhages, and stasis.

Franken (1959) discussed a fatality from chronic occupational exposure to methyl bromide. Considerable damage had occurred to the sensory

and motor spinal roots and ganglia. Large lesions were present in the cerebral and cerebellar cortex.

Kantarjian and Shaheen (1963) discussed eight non-fatal cases of chronic occupational exposure to methyl bromide. They pointed out that 2-6 other workers similarly exposed were apparently unaffected. The symptoms exhibited by the "eight" approximated the syndrome of polyneuropathy - numbness and heaviness of the legs, all; unsteadiness of gait, six; numbness of the hands, four; headache, coughing, anorexia, aches, etc., two-three. Deep reflexes were absent or sluggish. There were no severe systemic ill effects.

Drawneek et al (1964) commented that a serum level of 5 mg/100 ml of Br in workers using methyl bromide occupationally could induce in them a state of carelessness and euphoria not in keeping with the nature of their work.

Collins (1965) discussed a new case of non-fatal occupational methyl bromide poisoning and reviewed other cases, concentrating on the wide variety of disorders resultant from damage to the central nervous system.

Hine (1969) discussed four fatal and six non-fatal occupational methyl bromide poisonings occurring in California from 1957 to 1966.

Van Haaften (1969) reported the first known case of human poisoning from acetylene tetrabromide, $Br_2CHCHBr_2$. Hospitalization for severe hepatic damage resulted from breathing vapors during one work day. Apparently man reacts much more severely to this compound than do rats (see the next section).

Araki (1971) reviewed 14 cases of methyl bromide poisoning in Japan during 1964-1970. Tables 21 and 22 present the frequency of occurrence of various signs and symptoms.

Table 21. Symptoms of our fourteen cases

Symptom	Number of cases	%
Gait disturbance	12	86
Headache	11	79
Numbness of the extremities	9	64
Dizziness	7	50
Nausea, Vomiting	5	36
Speech disturbance	5	36
Blurred vision	3	21
Forgetfulness	3	21
Irritability	2	
Insomnia	2	
Emaciation	2	
Double vision	1	
Chills, Shivering	1	
Loss of libido	1	
Depression	1	
Anxiety	1	
Asthenopia	1	

Table 22. Signs manifested in our fourteen cases

Sign	Number of cases	%
Ataxia of gait, Incoordination	11	79
Contracted visual field (red perimetry)	8	57
Positive Romberg's sign	6	43
Exaggerated deep reflexes	6	43
Transient hypertension	5	36
Impaired superficial sensation	5	36
Hearing loss	4	29
Nystagmus	4	29
Muscular weakness	4	29
Hand tremor	4	29
Coma	2	
Intention tremor	2	
Impaired deep sensation	2	
Sluggish deep reflexes	2	
Muscular atrophy	2	
Inability to fix	2	
Skin rash	2	
Generalized convulsion	1	
Pathological reflexes	1	

Sax (1968) gave TLV's and Toxic Hazard Ratings for a number of bromohydrocarbons, as follows:

TLV (according to the ACGIH)

Methyl bromide	20 ppm (78 mg/m^3)
Ethyl bromide	200 ppm (892 mg/m^3)
Ethylene dibromide	25 ppm (190 mg/m^3)

Toxic Hazard Rating

Methyl bromide — Highest toxicity from acute local ingestion, inhalation, and irritation, also acute systemic inhalation and ingestion.

Sub-fatal toxicity from chronic systemic ingestion, inhalation, and skin absorption.

Ethyl bromide	Highest toxicity from acute systemic ingestion, inhalation, and skin absorption Sub-fatal toxicity from acute local irritant, chronic systemic ingestion, inhalation, and skin absorption.
Ethylene dibromide	Highest toxicity from acute local and systemic ingestion, inhalation, irritant, and skin absorption. Sub-fatal toxicity from chronic local irritant, chronic systemic ingestion, inhalation, and skin absorption.
Vinyl bromide	Sub-fatal toxicity from acute local inhalation, acute systemic inhalation, and chronic systemic inhalation.
Propyl bromide	Highest toxicity from acute local and systemic ingestion, inhalation, and irritation. Sub-fatal toxicity from acute systemic skin absorption. Unknown toxicity from chronic local or systemic contact.

From "Dangerous Properties of Industrial Materials" by N.I. Sax c 1975, 1968 by Litton Educational Publishing, Inc. Reprinted by permission of Van Nostrand Reinhold Company.

B. Birds and Mammals

1. Acute, subacute

Porritt et al (1952) subjected meadow mice to atmospheres of 4 or 8 g/l of methyl bromide; death occurred in three or two hours, respectively.

Rowe et al (1952) determined these LD-50's for ethylene dibromide

vapor exposure (g/kg): 0.055 for female rabbits, 0.079 for chicks, 0.110 for guinea pigs, 0.117 for female rats, 0.146 for male rats, and 0.420 for female mice. The effects noted in rats included, most importantly, lung irritation and hepatic injury; renal injury and central nervous system depression were also present.

Smyth, Jr. et al (1954) reported an oral, range finding LD-50 of 75 mg/kg for 1,4-dibromo-2-butene in rats.

Valade studied inhalation toxicity of methyl and ethyl bromide on dogs, guinea pigs, and rats. The LD-50's for 1/2-hour exposures were (g/m^3):10 for methyl, and > 100 for ethyl.

Davis and Hardcastle (1959) determined 24-hour median tolerance limits of bluegill sunfish (Lepomis macrochirus) and largemouth bass (Micropterus salmoides) for ethylene dibromide, 15-18 and 25-50 ppm, respectively (two sources of river water were used to hold the yearling specimens used).

Balander and Polyak (1962) reported an LD-50 of 1.54 mg/l for an inhalation dose of methyl bromide in white mice.

Sokolova (1962) exposed rats to 1 g/m^3 of methyl bromide. There was a reduction in oxygen requirement from 3-4 mg/l min. to 0.83-0.9 mg/l min. Hemoglobin, erythrocyte and leukocyte counts lended to increase, while serum catalase and cholinesterase tended to decrease.

Fuller and Morris (1962) introduced ethylene dibromide directly into the crops of young pullets and old hens, with equivalent results. Egg weight was reduced from a 1/2 mg/bird/day dose. Egg production was reduced by an 8 (but not 4) mg/bird/day dose. Production ceased from a 16 mg/bird/day dose. Recovery of production occurred 12 weeks after cessation of dosage, but egg weight recovery required 6-10 months.

Kutob and Plaa (1962) gave mice s.c. injections of di-, tri-, and tetrabromomethane. No hepatic damage was seen from 29 mmole/kg of the di-, 1.1 mmole/kg of the tri-, and only minimal from 0.05 mmole/kg of the tetra-. Most suffered damage from 4.4 mmole/kg of the tri-, and less than half suffered damage from 0.3 mmole/kg of the tetra-.

Kutob and Plaa (1962, pp. 354-61) reported these LD-50 values in mice for a s.c. dose and a 10-day observation period (mmole/kg): dibromomethane - 21.5, tribromomethane - 7.2, and tetrabromomethane - 0.9. They also determined LD-100 for the tetrabromo-, 1.5 mmoles/kg (six of seven mice died in 24 hours, the last in 48 hours). Based on the LD-50 values, the di- and tribromomethanes were classed as quick-acting, and the tetrabromo- as delayed action. It may be seen from the preceeding paragraph that a dose of the dibromo- greater than the LD-50 was still not hepatotoxic in mice.

Hollingsworth et al (1963) reported LD-0 of 0.6 g/kg and LD-100 of 1.6 g/kg for oral doses of acetylene tetrabromide in rats.

Dykan (1964) reported that rabbits exhibited central nervous system disorders from single exposures to 17-20 mg/l of dibromomethane or to 11-13 mg/l of tribromomethane.

Thompson (1966) reported a personal communication from H. A. U. Monro of an incident in which two horses died after drinking water contaminated with 404 ppm Br from exposure to methyl bromide.

Institóris et al (1967) reported these i.p. LD-50's for male mice (mg/kg): 1,4-dibromobutane, 300; 1,6-dibromohexane, 270.

Kakizaki (1967) reported a lethal dose for methyl bromide in rabbits of 130 mg/kg s.c. Characteristics of poisoning were paralysis of the hind limbs, cessation of drinking, and reduction of urine.

Leong and Torkelson (1970) reported an oral LD-50 of about 500 mg/kg for vinyl bromide in male rats. Vapor toxicity studies showed 100% mortality from 15 minutes exposure to 100,000 ppm (0.44 kg/m^3) and from seven hours exposure to 50,000 ppm (no fatalities from 1 1/2 hours exposure). No fatalities resulted from seven hours exposure to 25,000 ppm.

2. Chronic

Rowe et al (1952) reported that guinea pigs, monkeys, rabbits, and rats tolerated 25 ppm exposure to ethylene dibromide 7 h/d, 5 d/w, 24 weeks.

Rosenblum et al (1960) fed dogs for 6-8 weeks a diet which contained 35-150 mg/kg of Br from fumigation with methyl bromide. At the highest level gross obesity and lethargy resulted. No interference with methionine metabolism or symptoms of Br intoxication were seen.

Balander and Polyak (1962) reported that exposure of mice for two hours a day for 30 days to 0.15 mg/l of methyl bromide (1/10th of the LD-50) had no cumulative effects. No effects at all resulted from 20 days exposure to < 0.01 mg/l. For rabbits this threshold concentration was < 0.1 mg/l.

Dykan (1962) exposed rats for four hours a day for two months to 0.25 mg/l of di- and tribromomethane. Both, especially the tribromo-, caused disorders in the hepatic protein-prothrombin and glycogenesis functions, and also in the renal filtration capacity. When injected in 100-200 mg/kg doses daily for 10 days, both compounds proved detrimental to the liver and kidneys.

Kantarjian and Shaheen (1963) discussed a 1940 publication by D. D. Irish et al. They exposed rats and guinea pigs to 0.25 mg/l of methyl bromide for 7 1/2-8 hours/d, 6 d/w, for six months without gross symptoms

or histopathologic changes. Rabbits, however, showed paralysis of the extremities after 14-16 days (non-permanent). Monkeys were unaffected for at least five weeks, but some showed paralysis by three months (also non-permanent). Apparently some of the rabbits and monkeys were not affected at all during the course of treatment.

Thompson (1966) further elaborated on this publication by Irish. All animals died from an exposure to 0.85 mg/l lasting 12-24 hours. Daily exposures to 0.42 mg/l for eight hours was tolerated by rats for one week-five month periods, but poor growth and intoxication resulted. Guinea pigs were nearly unaffected after six months of the 0.42 mg/l dosage. Rabbits showed severe nervous response after only a few days. Even down to 0.13 mg/l (but not 0.065 mg/l) the rabbits developed paralysis.

Morris and Fuller (1963) demonstrated that ethylene dibromide had a measurable growth depressant effect on chicks when given in their diet at $\leq$ 40 ppm for one week.

Hollingsworth et al (1963) exposed a variety of animals to 14 ppm of acetylene tetrabromide 7 h/d, 5 d/w, 14-15 weeks. There was no unusual mortality. Other series of exposures involved 4 ppm for 26 weeks and 1.1 ppm for 28 weeks. The effects on growth, liver, and kidney weights are given in Tables 23-26.

At the highest dose the lungs of all species except guinea pigs showed signs of congestion, edema, and hemorrhage; rabbits and monkeys were especially effected. At the middle dose female guinea pigs showed all three effects, but only mice and male rats of the other animals showed any lung troubles. The low dose was uneventful medically.

TABLE 23.

Final Average Body, Liver and Kidney Weights From Rats that Received Repeated Seven-hour Exposures to Acetylene Tetrabromide Vapor

Vapor Conc., ppm	No. of Rats	Sex	No. of Exp.	Days on Expt.	Final Avg. Body Wt., g	Organ Weights			
						Liver		Kidney	
						g	g/100g	g	g/100g
Controls	10	M	0	100	322	7 50	2 33	2.12	0 66
14	10	M	70	100	287[d]	8 35[d]	2 93[a]	2.07	0 73
Controls	8	F	0	100	195	4 89	2 50	1.53	0 78
14	10	F	70	100	184[d]	5 77[c]	3 14[a]	1 56	0.85
Controls*	18	M	127	180	348	7 48	2 15	2.19	0 63
4	15	M	127	180	339	8 25[d]	2 43[b]	2 35	0 70
Controls*	18	F	128	181	209	5 25	2 51	1.48	0 71
4	17	F	128	181	197	5 78[c]	2 93[a]	1 54	0 78
Controls	17	M	131	190	386	8 52	2 21	2 24	0 58
1 1	16	M	131	190	368	8 60	2 35	2 14	0 58
Controls	19	F	132	191	234	5 64	2 48	1.52	0 65
1 1	20	F	132	191	237	5 90	2 42	1.52	0 69

*Air-exposed controls
**This weight is an average value for a group of four animals
(a) P=<0.001 (b) P=0.01 (c) P=0.05 (d) P=>0.06

TABLE 24.

Final Average Body, Liver and Kidney Weights from Guinea Pigs that Received Repeated Seven-hour Exposures to Acetylene Tetrabromide Vapor

Vapor Conc., ppm	No. of Rats	Sex	No. of Exp.	Days on Expt.	Final Avg. Body Wt., g	Organ Weights			
						Liver		Kidney	
						g	g/100g	g	g/100g
Controls	8	M	0	101	861	25 16	2 92	6 94	0 69
14	8	M	73	101	720[a]	26 61	3 70[a]	4 86	0.68
Controls	8	F	0	101	811	25 94	3 20	5 74	0.71
14	8	F	73	101	673[a]	24 53	3 64[a]	4 36	0.65
Controls*	8	M	129	182	926	29 55	3 20	5 92	0 64
4	8	M	129	182	744[a]	24 02	3 24	5 41	0 73[b]
Controls*	8	F	130	183	862	28 31	3 29	5 45	0.63
4	8	F	130	183	785	25 62	3 27	4 96	0 63
Controls*	8	M	133	192	852	26 6	3 14	5 84	0 69
1.1	8	M	133	192	807	26 48	3 28	5 14	0.64
Controls*	7	F	134	195	856	29 66	3 47	5 79	0 68
1.1	6	F	134	195	815	28 75	3 53	4 74	0 58

*Air-exposed controls
**This weight is an average value for a group of two animals
(a) P=<0 001 (b) P=0 004-0 006 (c) P=0 03

TABLE 25.

Final Average Body, Liver and Kidney Weights from Female Mice that Received Repeated Seven-hour Exposures to Acetylene Tetrabromide Vapor

Vapor Conc. ppm	No of Mice	No. of Exp.	Days on Expt.	Final Avg. Body Wt., g	Organ Weights			
					Liver		Kidney	
					g	g/100g	g	g/100g
Control	10	0	105	32	1 92	6 0	0 42	1 3
14	9	73	105	31	2 15[b]	6 9[c]	0 41	1 3
Control*	9	127	180	35	1 92	5 5	0 52	1 5
4	7	127	180	31[a]	1 73	5 6	0.45	1 5
Control*	9	130	189	27	1 51	5 6	0 34	1 3
1 1	6	130	189	27	1 44	5 4	0 32	1 2

*Air-exposed controls
(a) P=>0.1 (b) P=0 048 (c) P=<0 001

TABLE 26.
Final Average Body and Organ Weights from Rabbits and Monkeys that Received Repeated Seven-hour Exposures to Acetylene Tetrabromide Vapors

Vapor Conc. ppm	No. of Animals	Species	No. of Exp.	Days on Expt.	Final Avg. Body Wt., g	Organ Weights					
						Liver		Kidney		Testes	
						g	g/100g	g	g/100g	g	g/100g
Control	1M 1F	R*	0	106	3155	58 6	1 86	12 8	0 41	5 97	0 22
14	2M 2F	R	75	106	3120	90 8	2 90	14 3	0.46	4 09	0 14
Control*	2M 2F	R	129 130	182 183	3862	91 4	2.36	15 6	0 40	5 98	0 16
4	2M 2F	R	129 130	182 183	3775	112.7	2 95	15 8	0 42	5 12	0 16
Control*	2M 2F	R	135	196	3412	91 2	2.68	14 2	0.42	4 79	0 13
1.1	1M 1F	R	135	196	4490	108 5	2 42	17 6	0 39	5 51	0 13
Control	1M	M**	0	107	4690	100 4	2 14	19 6	0 42	8 55	0.18
14	1M	M	79	107	5460	138 1	2 53	21 6	0 40	4 22	0 08
Control	2F	M	136	198	4720	109 3	2 32	16 3	0 34	—	—
1.1	2F	M	136	198	4630	117 0	2.52	19 8	0 43	—	—

*R = Rabbits
**M = Monkeys
(*) Air-exposed controls

Tables 23-26 reprinted with permission from Amer. Ind. Hyg. Asso. J., 24:28-35. Copyright by the American Industrial Hygiene Association.

Dykan (1964) exposed rats to 2.5 mg/l of di- or tribromomethane for 10 days. Disorders were seen in the brain, liver, and kidneys. Threshold concentrations were determined as 0.23 and 0.05 mg/l for di- and tribromomethane, respectively.

Paustovskaya and Petrun (1969) exposed rats for up to four months to 0.01-0.1 mg/l, for 4 h/d, of tetrabromomethane. They observed disruption of carbohydrate metabolism at the fructose 1,6-diphosphate stage, inhibited tissue respiration, and hepatic/renal anaerobic glycolysis. Irritation of mucous membranes in the eyes and respiratory tract resulted even from a few days exposure at the minimum dose.

Leong and Torkelson (1970) exposed male rats to 10,000 ppm of vinyl bromide 7 h/d, 5 d/w, for four weeks. Lethargy was induced during each exposure. By the 15th exposure a definite slowing of growth compared to controls was noted. No other macro- or microscopic changes were seen.

In another series of experiments rats, rabbits, and monkeys were exposed to 250 or 500 ppm of vinyl bromide 6 hr/d, 5 d/w, for 24 weeks.

No statistically significant (at $p \leq 0.05$) external or internal changes resulted which were also dose-related.

3. Sensitization

4. Teratogenicity

5. Carcinogenicity

6. Mutagenicity

7. Behavioral effects

No mention was found in the literature of findings bearing on 3, 4, 5, or 6. Many of the bromohydrocarbons seem to have a lethargy-inducing property in man and animals. Most of the incidents of serious human exposure to methyl bromide mentioned alterations in behavior from brain damage.

C. Lower animals

In Table 27 is a compilation of scientific names in alphabetic order of insects and worms for whom there was found in the literature some indication of toxicity from bromohydrocarbons. Where the scientific name was accompanied by a common name, the latter was included in the table. Also in the table is the specific bromohydrocarbon, and the growth stage(s) of the insect if known. Because in most instances the toxic effect was an intentional one, and in some reports it was not clear what was meant by "toxic effect", and considering the general difficulty of determining percentage kills in large soil or stored products samples, it was decided not to further elaborate on most of the reports from which the table was compiled. The first reference to a particular insect - bromohydrocarbon combination is identified in the table, along with year of publication, by the "control number" assigned to the reference. Some of these references were secondary, the actual work having been reported prior to 1952.

Table 27. Insects Known to Be Susceptible to Bromohydrocarbons

Acanthoscelides obtectus (bean weevil); MeBr, $EtBr_2$; 10757 (1954)

Acarus siro (cheese, wheat mite); MeBr; 10793 (1966)

Achroia grisella (lesser wax moth); $EtBr_2$, 12155 (1958); EtBr, 14709 (1965)

Aedes aegypti (yellow-fever mosquito, eggs); $EtBr_2$; 12589 (1962)

Agriotes (wireworms); $EtBr_2$; 10717 (1956)

Amphimallon majalis (European chafer, all stages); $EtBr_2$; 11331 (1962)

Ancylostoma caninum (canine hookworm, larvae); $EtBr_2$; 12653 (1954)

Antagenus piceus (black carpet beetle); MeBr; 11318 (1962)

Anthrenus flavipes (carpet, furniture beetle, all stages); MeBr; 11318 (1962)

Anthrenus verbasci (varied carpet beetle); MeBr; 11318 (1962)

Aphelenchoides ritzema-bosi; 1,4-dibromopropyne; 11402 (1958)

Aphelenchus avenae (nematode); $EtBr_2$; 11199 (1971)

Argas persicus (tick); MeBr (EtBr not effective); 15335 (1955)

Ascaridia galli (chicken worm); 1,1-dibromoethane; 12355 (1952)

Ascaridia lineata (chicken worm, eggs); MeBr; 10734 (1955)

Atta cephalotes (ant); MeBr, $EtBr_2$; 10739 (1955)

Balaninus elephas (larvae); MeBr; 11047 (1954)

Baris lepidii; MeBr; 14944 (1965)

Belonolaimus (sting nematode); $EtBr_2$; 10734 (1955)

Belonolaimus longicaudatus Rau (sting nematode); MeBr; 11333 (1962)

Brachycerus (weevil); MeBr; 12491 (1963)

Brachytrupes membranaceus (DRU)(cricket); $EtBr_2$; 10747 (1954)

Calandra granaria (grain weevil); EtBr, PrBr, BuBr, PeBr, HexBr; 12936 (1953)

Caloglyphus krameri (mite); MeBr, $EtBr_2$; 14883 (1970)

Carpomyia vesuviana (Costa) (fruit fly, eggs,. larvae); $EtBr_2$; 10718 (1955)

Ceratitis capitata (Wied.) (Medit. fruit fly, larvae); $EtBr_2$; 10719 (1956)

Chilo agamemnon (corn borer, larvae); MeBr; 11172(1970)

Cochlicella barbara (snail); MeBr; 14237 (1965)

Conoderus amplicollis (Gyll.) (Gulf wireworm); $EtBr_2$; 10722 (1953)

Conoderus falli Lane (southern potato wireworm); $EtBr_2$; 11259 (1966)

Conoderus vespertinus F. (tobacco wireworm); $EtBr_2$; 11259 (1966)

Conotrachelus nenuphar Herbst (plum curculio, larvae); MeBr, $EtBr_2$; 11257 (1966)

Cryptolestes ferrugineus Stephens (rusty grain beetle); MeBr; 17849 (1967)

Cryptolestes turcicus Grouvelle; MeBr; 17849 (1967)

Dacus cucurbitae (Coq) (fruit fly, eggs, larvae); $EtBr_2$; 10718 (1955)

Dacus dorsalis - see write up following table (1954)

Dacus ferrugineus (Fab.) (fruit fly, eggs, larvae); $EtBr_2$; 10718 (1955)

Dacus zonatus (Saund) (fruit fly, eggs larvae); $EtBr_2$; 10718 (1955)

Dendroctonus engelmanni Hopkins (engelmann spruce beetle); $EtBr_2$; 10723 (1953)

Dendroctonus monticolae Hopkins (mountain pine beetle); $EtBr_2$; 10575 (1955)

Dendroctonus pseudotsugae Hopkins (Douglas fir beetle); $EtBr_2$; 10575 (1955)

Ditylenchus destructor (potato rot nematode); $EtBr_2$: 10734 (1955)

Ditylenchus dipsaci (eelworm); $EtBr_2$; 12706 (1958); MeBr, larvae, 11409 (1959)

Dorylaimus (nematode); MeBr; 11128 (1972)

Dyspessa ulula (Borkhausen) (carpenterworm moth); MeBr; 12491 (1963)

Ephestia elutella (tobacco moth, all stages); MeBr; 13415 (1959)

Ephestia kühniella (Medit. flour moth, larvae); MeBr; 12343 (1952)

Galleria mellonella (greater wax moth); $EtBr_2$; 12155 (1958); EtBr, 14709 (1965)

Glyptotermes dilatatus (live-wood termite); $EtBr_2$; 14812 (1970)

Gnorimoschema operculella (potato tuber moth); MeBr; 13728 (1958)

Helicotylenchus; $EtBr_2$; 10698 (1956)

Hemicycliophora parvana Tarjan (sheath nematode); MeBr; 11333 (1962)

Heterakis gallinae (from poultry, eggs); MeBr; 10734 (1955)

Heterodera avenae (cereal cyst-nematode); MeBr; 15766 (1970)

Heterodera glycines (soybean cyst-nematode); MeBr; 12928 (1958)

Heterodera marioni (rootknot eelworm); MeBr, $EtBr_2$; —

Heterodera rostochiensis Wollenweber (potato root eelworm, golden nematode of potatoes); MeBr; 12278 (1952); $EtBr_2$; 11054 (1953)

Hoplolaimus tylenchiformis (Daday) Andrassy (lance nematode); MeBr; 11333 (1962)

Lampetia equestris (narcissus bulb fly); MeBr; 13486 (1952)

Lasioderma serricorne (cigaret beetle, adults, larvae, eggs); $EtBr_2$; 12916 (1958)

Laspeyresia splendana (larvae); MeBr; 11047 (1954)

Leptinotarsa decemlineata Say (Colorado beetle); $EtBr_2$; 11241 (1971)

Limonius agonus (eastern field wireworm); $EtBr_2$; 11053 (1954)

Matsucoccus resinosae (scale of red pine); $EtBr_2$; 13425 (1959)

Meloidogyne hapla Chitwood (root-knot nematode); $EtBr_2$; 10698 (1956)

Meloidogyne incognita var. acrita (root-knot nematode); $EtBr_2$; 10756 (1954)

Meloidogyne javanica (root-knot nematode); MeBr; 13399 (1959)

Musca domestica L. (house fly, larvae); $EtBr_2$; 11418 (1962)

Nippostrongylus muris (larvae); BuBr; 10585 (1955)

Ophiobolus graminis (take-all); MeBr; 15766 (1970)

Oryzaephilus surinamensis (sawtoothed grain beetle); MeBr, $EtBr_2$; 10757 (1954)

Ostrinia nubilalis (corn borer, larvae); MeBr; 11172 (1970)

Oulema melanopa (cereal leaf beetle); MeBr; 14887 (1970)

Panagrellus redivivus (nematode, pre-adult); 1,4-dibromopropyne; 11402 (1958)

Paratylenchus (pin nematode); $EtBr_2$; 10559 (1961)

Periplaneta americana (American cockroach); $EtBr_2$; 14204 (1964)

Phylloxera vitifoliae - see write up following table (1962)

Pleocoma (fruit root grub); $EtBr_2$; 17851 (1970)

Popillia japonica (Japanese beetle, grubs); $EtBr_2$; 14553 (1958)

Pratylenchus penetrans (root-lesion nematode); MeBr, $EtBr_2$; 14569 (1961)

Pratylenchus pratensis; MeBr; 12935 (1953)

Pratylenchus vulnus (root-lesion nematode); $EtBr_2$; 10756 (1954)

Quadraspidiotus perniciosus Comst. (San Jose scale); MeBr; 17843 (1967)

Radopholus similis (burrowing nematode); $EtBr_2$; 13931 (1961)

Rhagoletis mendax (blueberry maggot); MeBr, $EtBr_2$; 14349 (1970)

Rhagoletis pomonella (apple maggot); MeBr, $EtBr_2$; 12619 (1962)

Rhyzopertha dominica (lesser grain borer); MeBr; $EtBr_2$; 10757 (1954)

Rotylenchulus reniformis (nematode); MeBr; 15188 (1960)

Sitophilus granarius (granary weevil); MeBr; 13476 (1952); $EtBr_2$; 10757 (1954)

Sitophilus oryzae (rice weevil); MeBr, $EtBr_2$; 10757 (1954)

Stegobium paniceum (drugstore beetle); MeBr, $EtBr_2$; 10757 (1954)

Syngamus trachea (from poultry, eggs); MeBr; 10734 (1955)

Tarsonemus myceliophagus (mite); MeBr; 17838 (1966)

Tenebrio molitor (larvae, adults); MeBr; 13759 (1959)

Tenebroides mauritanicus (cadelle, black grain gnawer); MeBr; 13476 (1952); $EtBr_2$; 14197 (1961)

Theba pisana (Müller) (white garden snail); MeBr; 14237 (1965)

Tribolium castaneum (red flour beetle); MeBr; 14243 (1965)

Tribolium confusum (confused flour beetle); MeBr, $EtBr_2$; 10757 (1954)

Trichodorus christiei Allen (stubby-root nematode); MeBr; 11333 (1962)

Trichostrongylus axei (nematode); MeBr; 15811 (1965)

Trichostrongylus colubriformis (nematode); MeBr; 15811 (1965)

Trogoderma granarium (khapra beetle); MeBr; 15091 (1952)

Tylenchorhynchus martini Fielding (stylet nematode); MeBr; $EtBr_2$; 10699 (1956)

Tylenchulus semipenetrans Cobb (citrus nematode); MeBr, propargyl bromide; 10772 (1966)

Tyrophagus (lintneri) (mite); MeBr; 14488 (1962)

Tyrophagus putrescentiae (mite); MeBr; 12310 (1966)

Xiphinema index (dagger nematode); MeBr; 15506 (1971)

Zabrotes pectoralis (Mexican bean weevil); MeBr, $EtBr_2$; 10757 (1954)

Cockroach; MeBr; 15130 (1961)

Fly; MeBr; 15130 (1961)

Pink bollworm (cotton); MeBr; 12776 (1952)

Termite; MeBr; 15130 (1961)

MeBr - methyl bromide, EtBr - ethyl bromide, $EtBr_2$ - 1,2-ethylene dibromide, PrBr - n-propyl bromide, BuBr - n-butyl bromide, PeBr - n-pentyl bromide, HexBr - n-hexyl bromide

Hinman (1954) tested the effectiveness of a variety of compounds against day-old eggs, and third-instar larvae of the oriental fruit fly Dacus dorsalis. Exposure time to the vapors was two hours at 24°C. Results are in Table 28.

Table 28. Effectiveness of various compounds used as fumigants against naked eggs and third-instar larvae of the oriental fruit fly. Milligrams per liter giving 50 and 95 per cent mortality 48 hours after exposure.

Compound	Eggs		Larvae	
	LD-50	LD-95	LD-50	LD-95
Halogenated Aliphatic Hydrocarbons, Saturated				
Bromides				
Methane, dibromo- (methylene dibromide)	18	38	192	218
Bromoform	42	190	>305	>305
Carbon tetrabromide (solid)	>250	>250	75	>250
Ethane, bromo-[1]	121	194	201	>300
Ethane, 1,1-dibromo-	>213	>213	>213	>213
Ethane, 1,1,2,2-tetrabromo-	>151	>151	>151	>151
Propane, 1-bromo- (*n*-propyl bromide)[1]	52	93	88	190
Propane, 2-bromo- (*sec*-propyl bromide)	>202	>202	>202	>202
Propane, 1-bromo-2-methyl- (isobutyl bromide)	>195	>195	>195	>195
Propane, 2-bromo-2-methyl-(*tert*-butyl bromide)	>188	>188	164	>188
Propane, 1,2-dibromo-	0.1	18	0.3	1.8
Propane, 1,3-dibromo-[1]	<5.1	5.5	0.1	18
Propane, 1,2-dibromo-2-methyl- (isobutylene bromide)	>179	>179	33	130
Butane, 1-bromo-2-methyl- (*pri-act*-amyl bromide)	>188	>188	54[1]	215[1]
Butane, 1-bromo-3-methyl- (isoamyl bromide)	>187	>187	65	131
Butane, 1-bromo-2-ethyl-	>173	>173	48	>173
Butane, 2-bromo- (*sec*-butyl bromide)	>194	>194	>194	>194
Butane, 2-bromo-2-methyl- (*t*-amyl bromide)	>183	>183	>183	>183
Butane, 1,2-dibromo-	5.5	8.7	4.1	7.8
Butane, 1,3-dibromo-	>184	>184	14.6	31
Butane, 1,4-dibromo-	40	>188	21	45
Butane, 2,3-dibromo-	>170	>170	3.3	>170
Pentane, 1-bromo- (*n*-amyl bromide)	38	81	44	92
Pentane, 2-bromo- (*sec*-amyl bromide)	>162	>162	88	>162
Pentane, 1,2-dibromo-	>170	>170	0.6	1.6
Pentane, 1,4-dibromo-	>162	>162	>162	>162
Pentane, 1,5-dibromo-	3.2	>174	>174	>174
Pentane, 2,4-dibromo-	>156	>156	>156	>156
Hexane, 1-bromo- (*n*-hexyl bromide)	>119	>119	>119	>119
Hexane, 1-bromo-2-ethyl-	>107	>107	>107	>107
Hexane, 1-bromo-3,5,5-trimethyl-	>105	>105	>105	>105
Hexane, 2,5-dibromo-	>150	>150	>150	>150
Heptane, 1-bromo- (*n*-heptyl bromide)	16	>116	40	>116
Heptane, 2-bromo- (*sec*-heptyl bromide)	>143	>143	>143	>143
Octane, 1-bromo- (*n*-octyl bromide)	13	21	>113	>113
Octane, 2-bromo- (*sec*-octyl bromide)	>111	>111	>111	>111
Nonane, 1-bromo- (*n*-nonyl bromide)	>110	>110	>110	>110
Decane, 1-bromo- (*n*-decyl bromide)	>109	>109	>109	>109
Dodecane, 1-bromo- (*n*-dodecyl bromide)	>100	>100	>100	>100
Tetradecane, bromo-	>97	>97	>97	>97
Hexadecane, bromo-	>96	>96	>96	>96
Halogenated Aliphatic Hydrocarbons, Unsaturated				
Propene, 1 bromo-	>146	>146	>146	>146
Propene, 1,1-dibromo-	135	>191	55	>191
Halogenated Cycloparaffins				
Cyclopentane, bromo-	9	16	7.5	14
Cyclohexane, bromo-	>130	>130	>130	>130
Cyclohexane, 1,2-dibromo-	>173	>173	>173	>173

[1] Mortality at 24 hours.

For comparison the author used these data from Balock and Lindgren (1951): methyl bromide had LD-95 for eggs at 25, and larvae at 19 mg/l; ethylene dibromide had LD-95 for eggs at 0.8, and larvae at 0.6 mg/l.

Monro et al (1961) in 1953 began a study on two wild and one laboratory strain of the granary beetle Sitophilus granarius which involved treating them with methyl bromide vapor and breeding new generations from survivors of > LD-50 (or higher) doses. Exposure was standardized at five hours, 25°C, and 70% R.H.; only adults were used. The results are in Figures 6 and 7. The "A" selected strains were begun after 1956 from survivors of > LD-50 doses, whereas the selected non-A strains were survivors of > LD-75 doses; apparently the wider gene pool available to the "A"'s increased resistance faster. The non-selected groups were the controls for the experiment, and showed no inherent ability to increase resistance. Discontinuance of selection did not cause reversion to the 1953 resistance level. At the time of writing, the LW strain had shown a 24% and the MW strain a 41% increase in body weight. Simultaneous experiments with Tribolium confusum and Tenebroides mauritanicus did not generate much increase in resistance.

An A strain which was 5.5 times more resistant to an LD-50 dose of methyl bromide as the normal was also shown to be 3 times more resistant to an LD-50 dose of ethylene dibromide.

Rammer and Stafford (1962) exposed first-instar female nymphs of Phylloxera vitifoliae (Fitch) to a variety of brominated propanes for four hours at 21°C, or for eight hours at 21°C in the presence of soil. Some studies were also done at 13 and 30°C. The results are in Tables 29-33 and Figure 9.

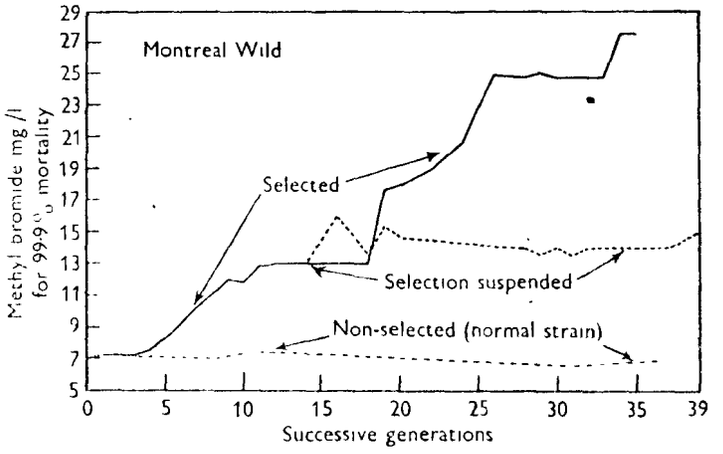

Fig. 7 Effect of suspension from selection by methyl bromide of one portion of a population of *S. granarius*, compared with continuance of selection of the remainder.

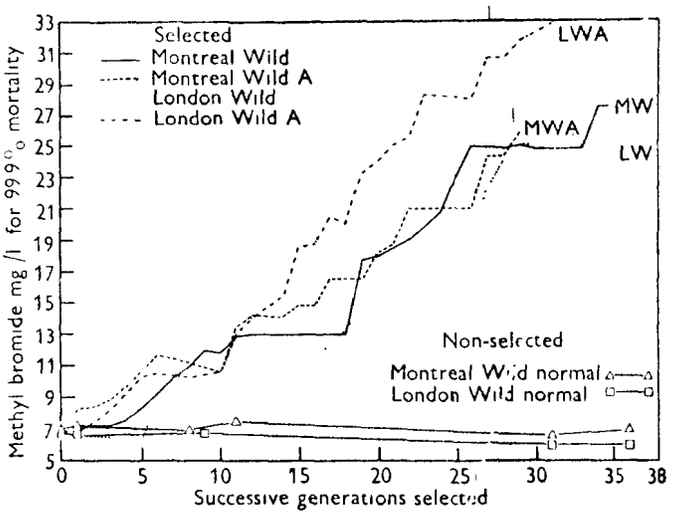

Fig. 6 History of selection of increased adult tolerance to methyl bromide fumigation of four stocks of *S. granarius* compared with normal non-selected stock.

Figures 6 & 7 reprinted with permission from Ann. Appl. Biol. 49:371-377 (1961). Copyright the Association for Applied Biologists.

Table 29. Toxicity of various bromopropanes to grape phylloxera nymphs.[a]

Propane	ED_{50} Mg/L[b]		ED_{50} Micro-moles/L.	Relative Vapour Saturation at ED_{50}	ED_{90} Mg/L	Slope
1,3-Dibromo-	0·44	a	2·18	0·010	0·55	12·6
1,2,3-Tribromo-	1·42	b	5·06	0·35	1·95	9·0
1,2-Dibromo-3-chloro-	1·95	c	8·25	0·19	2·68	9·3
1,2-Dibromo-	12·1	d	59·9	0·18	21·2	5·3
1-Bromo-	14·5	e	117·9	0·019	19·7	10·0

[a] 70° F., 4 hours' exposure, no soil.

[b] Significant differences between means at the 1% level are indicated when computed values have no letters in common. Duncan's Multiple Range Test (1955).

Lindgren and Vincent (1962) studied the effect of moisture content of various commodities on mortality of Tribolium confusum and Sitophilus oryzae from application of a fixed dose of methyl bromide. The results

Table 30.-Effect of temperature on the toxicity of 1,3-dibromopropane to grape phylloxera nymphs. (4 hours' exposure without soil.)

Temperature (° F.)	ED_{50} Mg./L.[a]	Relative Vapor Saturation at ED_{50}	ED_{90} Mg./L.	Slope
55	0.50	0.036	0.66	10.7
70	0.44	0.019	0.55	12.6
85	0.38	0.010	—[b]	2.0

[a] No significant difference between mean effective doses at the 5% level. Duncan's Multiple Range Test (1955).

[b] ED_{90} is not reported owing to the divergence of the highest concentration from the general trend set by the other three concentrations. The ED_{50} and slope are based on the three lowest concentrations.

Table 31. Toxicity of various bromopropanes to grape phylloxera nymphs.[a]

Propane[b]	ED_{50} per 87 G Dry Soil: Mg.[c]	ED_{50} per 87 G Dry Soil: Micromoles	ED_{90} in Mg./87 G. Dry Soil	Slope
1,3-Dibromo	5.65 a	28.0	6.66	17.6
1-Bromo-	20.1 b	163.4	50.5	3.2
1,2-Dibromo-	26.1 c	139.3	31.5	15.5
1,2-Dibromo-3-chloro-	2040.0 d	8630.0	3250.0	6.4

[a] 70° F., 8 hours' exposure, 100 grams soil of 15% moisture.

[b] The ED_{50} for 1,2,3-tribromopropane was greater than 15,128 mg.

[c] Significant differences between means at the 1% level are indicated when compared values have no letters in common. Duncan's Multiple Range Test (1955).

Table 32.-Effect of soil temperature on the toxicity of 1,3-dibromopropane to grape phylloxera nymphs.[a]

Temperature (° F.)	ED_{50} in Mg./87 G. Dry Soil[b]	ED_{90} in Mg./87 G. Dry Soil	Slope
55	14.50 a	16.20	26.7
70	5.65 b	6.66	17.6
85	1.22 c	1.82	7.7

[a] 8 hours' exposure, 100 grams soil of 15% moisture.

[b] Significant differences between means at the 1% level are indicated when compared values have no letters in common. Duncan's Multiple Range Test (1955).

Table 33.-Effect of soil moisture on the toxicity of 1,3-dibromopropane to grape phylloxera nymphs. (8 hours' exposure, 70° F.)

Moisture Content[a] (%)	ED_{50} in Mg./87 G. Dry Soil[d]	ED_{90} in Mg./87 G. Dry Soil	Slope
15[b]	5.65	6.66	17.6
22[c]	2.58	3.13	15.5

[a] ED_{50} at 5% moisture content (91.6 grams) was greater than 5346 mg.

[b] 100 g. soil

[c] 100.2 g. soil.

[d] Duncan's Multiple Range Test (1955) indicates a significant difference between means at the 1% level

Tables 29-33 reprinted with permission from J. Econ. Entomol. 55:203-11 (1962). Copyright by the Entomological Society of America.

are in Table 34. Regardless of commodity or moisture content, 100% kills of T. confusum were obtained if the concentration (actual) x time value (CT) was greater than 75 or if at least 50% of the applied dose (CL-50) remained unabsorbed by the commodity for four hours; corresponding values for S. oryzae were: CT over 31, and CL-50 over 2.5 hours.

Moje (1963) tested $CH_3(CH_2)_nCH_2Br$, n = 2-9, against citrus nematode larvae. He found that toxicity increased by a factor of 2.45 for each additional CH_2 group. Cyclohexyl and cyclopentyl bromides were less toxic than the n = 2 compound.

Harein and Soles (1964) tested crotyl bromide (86% 1-bromo-2-butene, 14% 3-bromo-1-butene) and 1,2,3-tribromopropene against the adults of Tribolium confusum, Oryzaephilus surinamensis, Lasioderma serricorne, and the larvae of Attagenus piceus. The results are given in Table 35.

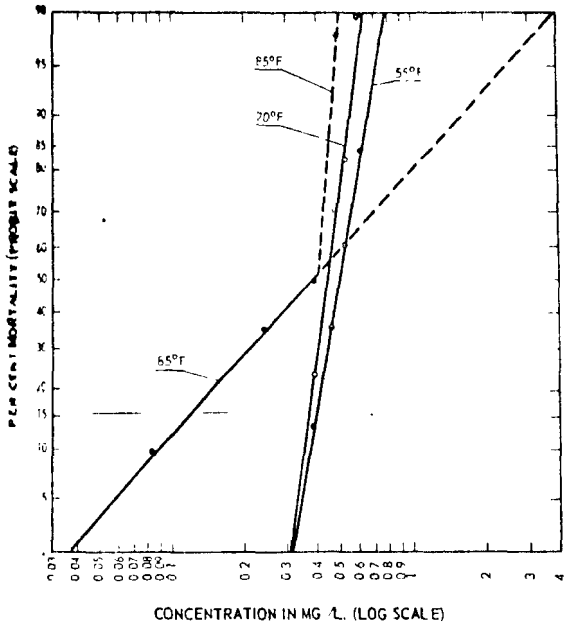

Fig. 9. Effect of temperature on the mortality of grape phylloxera nymphs fumigated with 1,3-dibromopropane for 4 hours in the absence of soil.

Table 34.—Concentrations found and mortalities of adults of *Tribolium confusum* and *Sitophilus oryzae* obtained when Fumigating various commodities at different moisture contents with methyl bromide in 10-liter recirculation chambers Exposure: 24 hours at 70° F. Load: 75%.

Commodity	Moisture Content (%)	Mean Average[a] Concentration (mg/l)	d.t.[b]	c.t.[c]	CL-50[d] (hrs)	Per Cent Kill of Adults *T. confusum*	*S. oryzae*
Barley	9.6	7.1	192	170	>24.0	100.0	100.0
	12.0	5.4	192	130	>24.0	100.0	100.0
	15.6	5.0	192	120	22.0	100.0	100.0
Rice	9.0	6.2	192	149	24.0	100.0	100.0
	12.2	5.7	192	137	23.0	100.0	100.0
	16.6	2.8	192	67	3.3	100.0	100.0
Wheat, whole kernel	11.0	5.6	192	134	21.0	100.0	100.0
	13.0	4.1	192	98	11.0	100.0	100.0
	15.0	2.9	192	70	3.8	100.0	[illegible]
Wheat, cracked	9.9	2.2	192	53	1.5	9.6	100.0
	13.0	2.2	192	53	1.1	8.5	100.0
	16.4	2.1	192	50	0.8	2.9	100.0
Oats	10.8	3.7	192	89	5.9	100.0	100.0
	12.5	3.3	192	79	4.0	100.0	100.0
	15.8	2.3	192	55	1.4	51.4	100.0
Popcorn	11.2	4.3	192	103	10.5	100.0	100.0
	13.1	2.5	192	60	3.0	96.0	100.0
	15.2	1.6	192	38	1.4	17.2	100.0
Small white beans	11.5	2.9	192	70	4.3	100.0	100.0
	13.4	1.9	192	46	2.6	97.2	100.0
	14.9	1.4	192	34	1.7	0.5	100.0
Green split peas	9.3	3.3	192	79	5.4	100.0	100.0
	10.8	2.0	192	48	3.0	51.0	100.0
	12.6	0.6	192	14	0.5	0	0
Great northern beans	10.2	3.1	192	74	5.1	100.0	100.0
	12.3	1.3	192	31	1.6	0	100.0
	13.7	1.0	192	24	0.8	0	100.0
Corn	11.0	3.1	192	74	3.8	99.6	100.0
	13.0	2.3	192	55	1.7	9.4	100.0
	15.0	1.8	192	43	1.0	0	100.0
Blackeye beans	9.9	3.8	192	91	9.0	100.0	100.0
	11.2	1.2	192	29	2.0	0	97.1
	13.2	0.4	192	10	0.2	0	0
Milo	10.5	2.8	192	67	2.5	72.7	100.0
	12.5	2.3	192	55	1.5	52.8	100.0
	15.0	1.8	192	43	0.4	0	100.0
Pinto beans	11.2	2.4	192	58	3.4	98.1	100.0
	12.4	1.3	192	31	1.4	0	100.0
	14.4	1.1	192	26	0.6	0	95.7
Lentils	10.9	2.6	192	62	3.8	98.0	100.0
	13.1	0.8	192	19	0.8	0	14.0
	14.1	0.6	192	14	0.4	0	1.4
Flax	10.7	1.0	192	24	0.8	0.5	88.0
	13.0	0.6	192	14	0.4	0	32.1
	14.8	0.5	192	12	0.3	0	3.9
No load (empty chamber)	—	1.8	48	44	>24.0	0.4	100.0
	—	2.8	72	67	>24.0	80.0	100.0
	—	3.0	79	72	>24.0	100.0	100.0
	—	3.9	96	94	>24.0	100.0	100.0

[a] ΣMC×T/ΣT = Mean average concentration, where MC is equal to mean concentration for each time interval over which MC is computed, and T is equal to [illegible]

[b] d.t. [illegible]

[c] c.t. = Mean average concentration (mg/l) × time (hrs).

[d] CL-50 = Time required for concentration loss of 50% of dosage applied.

Table 35.-Toxicity of 2 chemicals to 4 species of stored-product insects fumigated 24 hours at 80° ± 4° F. and at a relative humidity of 78 ± 18% in 19.5-liter bottles.

Insect	Dosage (mg/l)					
	LD_{50}	LD_{50} fiducial limits		LD_{95}	LD_{95} fiducial limits	
		Low	High		Low	High
			1,2,3,-Tribromopropene			
Confused flour beetle	0.54	0.52	0.56	0.89	0.83	0.96
Saw-toothed grain beetle	.20	.17	.23	.35	.26	.47
Cigarette beetle	.35	.32	.37	.51	.45	.58
Black carpet beetle	1.18	1.04	1.35	2.39	1.93	2.98
			Crotyl bromide			
Confused flour beetle	2.32	2.18	2.47	2.97	2.67	3.31
Saw-toothed grain beetle	1.32	1.27	1.38	1.90	1.76	2.07
Cigarette beetle	1.03	1.00	1.08	1.49	1.41	1.59
Black carpet beetle	3.92	3.55	4.33	6.15	5.11	7.40

D. Plants

Table 36 is similar to Table 27 dealing with toxicity of bromohydrocarbons to plants and fungi rather than insects. Many of the entries resulted as incidental findings from studies of insect toxicology. References to delayed germination of seeds, and fruit damage were omitted.

Table 36. Plants and Fungi Known to be Susceptible to Bromohydrocarbons

Alternaria solani; MeBr; 17879 (1959)

Armillaria mellea (citrus root rot); MeBr; 15637 (1969)

Aspergillus parasiticus; MeBr; 14731 (1970)

Botrytis cinerea (soil fungus); MeBr; 12252 (1953)

Ceratostomella fimbriata (sweet potato black rot); $EtBr_2$; 10734 (1955)

Chenopodium album (fat hen); MeBr; 10782 (1954)

Colletotrichum atramentarium (soil fungus); $EtBr_2$; 13492 (1952)

Corticium solani (soil fungus); $EtBr_2$; 13492 (1952)

Cylindrocladium scoparium (pine root-rot); MeBr; 11093 (1971)

Cyperus compressus (annual sedge); MeBr; 11420 (1962)

Cyperus esculentus (yellow nut sedge); MeBr; 11420 (1962)

Cyperus rotundus (nut grass, Topalak weed); MeBr; 15657 (1969)

Digitaria sanguinalis (crab grass); MeBr; 11420 (1962)

Fusarium bulbigenum lycopersici (tomato wilt); MeBr; 14334 (1968)

Fusarium lini (soil fungus); $EtBr_2$; 13492 (1952)

Fusarium oxysporum var. auriantiacum (soil fungus); $EtBr_2$; 13492 (1952)

Fusarium oxysporum f. niveum (soil fungus); MeBr; 17785 (1954)

Fusarium vasinfectum (cotton wilt); $EtBr_2$; 10734 (1955)

Gallium aparine (goose grass); MeBr; 11420 (1962)

Gallium asprellum (rough bed straw); MeBr; 11420 (1962)

Hemileia vastatrix; MeBr; 11094 (1971)

Lepidium sativum; MeBr; 14885 (1969)

Lepidium virginicum (Virginia peppercress); MeBr; 10782 (1954)

Linaria canadensis (blue toad flax); MeBr; 11420 (1962)

Mycelia sterilia; MeBr; 14885 (1969)

Orobanche ludoviciana var. cooperi (broomrape); MeBr; 13775 (1959)

Orobanche ramosa (broomrape); MeBr; 13732 (1958)

Oxalis latifolia; MeBr; 14722 (1964)

Panicum repens (torpedograss); MeBr; 12562 (1963)

Penicillium rubrum; MeBr; 14731 (1970)

Phytophthora cactorum (soil fungus); $EtBr_2$; 13492 (1952)

Phytophthora cinnamomi (soil fungus); MeBr; 12252 (1953)

Phytophthora citrophthora (soil fungus); MeBr; 12252 (1953)

Phytophthora cryptogea (soil fungus); $EtBr_2$; 13492 (1952)

Phytophthora fragariae (strawberry red stele disease); MeBr; 13017 (1957)

Phytophthora parasitica var. nicotianae (tobacco black shank); MeBr; 12879 (1956)

Plasmodiophora brassicae (cabbage clubroot); MeBr; 13457 (1960)

Polygonum aviculare (wireweed); MeBr; 10782 (1954)

Poria hypolaleritia (tea root-rot); $EtBr_2$; 11092 (1969)

Pythium ultimum (soil fungus); $EtBr_2$; 13492 (1952)

Rhizoctonia solani (soil fungus); MeBr; 17784 (1953)

Saccharum spontaneum (grass); MeBr; 10748 (1956)

Sclerotinia homeocarpa (soil fungus); MeBr; 12252 (1953)

Sclerotinia minor; MeBr; 14885 (1969)

Sclerotinia sclerotiorum (soil fungus); $EtBr_2$; 13492 (1952)

Sclerotium bataticola; MeBr; 13399 (1959)

Sclerotium delphinii (soil fungus); MeBr; 17784 (1953)

Sclerotium rolfsii; MeBr; 10738 (1955)

Solanum opacum (black nightshade); MeBr; 10782 (1954)

Spergula arvensis (spurge); MeBr; 11420 (1962)

Synchytrium endobioticum; MeBr; 14145 (1970)

Thielaviopsis basicola (black root-rot); MeBr; 13459 (1959)

Tilletia foetida (wheat bunt); MeBr; 14335 (1968)

Trifolium glomeratum (cluster clover); MeBr; 10782 (1954)

Urocystis tritici (wheat flag smut); MeBr; 14336 (1968)

Verticillium albo-atrum (soil fungus); $EtBr_2$; 13492 (1952)

Verticillium dahliae (soil fungus); $EtBr_2$; 13492 (1952)

Waitea circinata (pine root-rot); $EtBr_2$; 17850 (1971)

Beans (plants); 1,4-dibromo-2-butene, 1,4-dibromo-2-butyne; 11426 (1957)

Beets (seeds); $EtBr_2$; 10749 (1955)

Broccoli (seeds); $EtBr_2$; 10749 (1955)

Carnation; MeBr, $EtBr_2$; 12931 (1953)

Carrots (seeds); $EtBr_2$; 10749 (1955)

Celery (seeds); $EtBr_2$; 10749 (1955)

Clover (seeds); $EtBr_2$; 10749 (1955)

Corn (seeds); $EtBr_2$; 10749 (1955)

Cucumbers (plants); 1,4-dibromo-2-butene, 1,4-dibromo-2-butyne; 11426 (1957)

Eggplant (seeds); $EtBr_2$; 10749 (1955)

Gladiolus; MeBr; 10738 (1955)

Groundnut; MeBr; 10701 (1955)

Lettuce (seeds); $EtBr_2$; 10749 (1955)

Lime; MeBr; 10762 (1954)

Maize (plants); 1,4-dibromo-2-butene, 1,4-dibromo-2-butyne; 11426 (1957)

Morning glory (plants); 1,4-dibromo-2-butene, 1,4-dibromo-2-butyne; 11426 (1957)

Mushroom; MeBr; 17838 (1966)

Mustard (seeds); $EtBr_2$; 10749 (1955)

Narcissus; MeBr; 14236 (1965)

Nutgrass; MeBr; 10727 (1955)

Oats (seeds); $EtBr_2$; 10749 (1955)

Oats and wild oats (plants; 1,4-dibromo-2-butene, 1,4-dibromo-2-butyne;
11426 (1957

Onion (seeds); $EtBr_2$; 10749 (1955)

Orange; MeBr; 10762 (1954)

Pea (plants); 1,4-dibromo-2-butene, 1,4-dibromo-2-butyne; 11426 (1957)

Potato; MeBr; 12273 (1952)

Radish (plants); 1,4-dibromo-2-butene, 1,4-dibromo-2-butyne; 11426 (1957)

Rutabaga (seeds); $EtBr_2$; 10749 (1955)

Rye (seeds); $EtBr_2$; 10749 (1955)

Rye (plants); 1,4-dibromo-2-butene, 1,4-dibromo-2-butyne; 11426 (1957)

Spinach (seeds); $EtBr_2$; 10749 (1955)

Tobacco (seeds); MeBr; 16482 (1957)

Tomato (seeds); $EtBr_2$; 10749 (1955)

Turnip (seeds); $EtBr_2$; 10749 (1955)

Cobb (1956) reported that susceptibility of seeds to methyl bromide generally increased with moisture content; temperature and exposure time were also factors. Even though some seeds survive and germinate, the resultant sprouts may be weak and die soon or produce stunted plants.

Martin et al (1956) found that orange seedlings absorbed Br from soil treated with ethylene dibromide. Concentrations of Br in the leaves of 0.17, 0.33, 0.40, 1.3, and 1.8% produced growth reductions of 12, 22, 31, 57, and 90%, respectively. Leaf Br concentrations of 2.5 or 1.5% in carrots or lima beans, respectively, were not deleterious to growth.

Whitney et al (1958) studied the toxic effect of fumigation with methyl bromide on barley, corn, grain sorghum, oats, and wheat seeds. They found that little or no injury resulted when all of the following conditions existed: seed moisture < 12%, dosage < 32 kg/m^3, exposure < 24 hours, and temperature = 27°C. Relative tolerances of the seeds examined were: oats > barley > grain sorghum > corn > wheat.

Viel and Giban (1958) found that an application of 200 g/m^2 of ethylene bromide, the usual for nematode fumigation, was harmless to the growth of tomatoes. Retardation resulted from a dose 10 times that amount.

Blackith and Lubatti (1960) reported that moisture content of seeds was a greater factor in damage from methyl bromide fumigation the greater the oil content of the seeds. The oil also increased germination delays by storing the fumigant in solution.

Blackith and Lubatti (1965) reported the results of a six year study on germination ability of seeds containing an 8-18% water content after fumigation with 0-1200 mg/l for one hour (or equivalent) of methyl bromide. Their results are in Table 37.

Table 37.

Percentage germination capacity (means of 400 seeds tested on each occasion) of fumigated, stored cereals

Dosage of methyl bromide in mg.h./l.

Cereals	Moisture content, %	Controls (unfumigated seed) Storage for		(Concentration × time product) 600 Storage for		1200 Storage for	
		3 years	6 years	3 years	6 years	3 years	6 years
Wheat (Peko)	8	93·0	91·3	92·0	93·3	93·0	88·8
	11	89·5	87·0	94·5	93·0	85·0	90·0
	14	88·5	90·5	24·3	25·0	25·5	20·8
	18	31·8	14·5	6·8	0	1·0	0
Wheat (Atle)	8	95·0	93·0	93·0	92·5	96·0	92·0
	11	91·8	85·0	92·0	88·5	63·5	58·0
	14	92·8	88·3	40·5	35·8	41·8	30·5
	18	87·0	0	0·3	0	3·5	0
Oats (Star)	8	36·5	85·0	85·8	80·0	82·0	76·0
	11	84·5	82·0	82·5	77·0	69·3	69·0
	14	82·5	65·0	60·3	46·8	36·5	23·3
	18	0·5	0	7·3	0	1·3	0
Oats (Blenda)	8	98·0	96·8	98·5	96·5	96·3	97·0
	11	99·3	97·8	94·5	94·5	97·5	93·0
	14	96·0	96·0	89·0	86·0	75·5	55·3
	18	48·8	7·8	3·0	0	1·8	0
Barley (Procter)	8	96·5	95·3	97·3	97·3	97·3	95·3
	11	96·5	94·0	95·8	94·0	96·3	92·0
	14	95·0	93·8	81·5	81·3	81·8	85·0
	18	0	0	52·8	17·8	0	0
Barley (Herta)	8	97·5	93·5	99·0	93·8	98·3	94·5
	11	99·3	96·5	99·3	92·0	81·3	83·8
	14	95·5	84·7	75·5	67·8	75·3	70·3
	18	4·3	0	0	0	0	0
Rye (Winter)	8	91·8	28·0	98·3	27·8	97·3	35·7
	11	99·0	27·5	93·0	13·5	72·3	9·3
	14	69·5	4·3	39·0	0·8	44·8	0
	18	23·3	0	23·8	0	23·5	0
Maize (W268)	8	—	94·8	—	96·5	—	96·8
	11	—	94·0	—	65·5	—	48·0
	14	—	73·0	—	32·3	—	2·7
	18	—	0	—	0	—	0

Wilson and Norris (1966) applied ethylene dibromide to a soil each year for nine years at 11 ml/m^2. Average crop yields for the last three years as a percentage of the yields from an untreated soil were: onions - 64, potatoes - 44, carrots - 102, celery - 106, beets - 83, lettuce - 79, radish - 113, and spinach - 92. The reduced yields of onions and potatoes resulted from poorly growing plants, not smaller sized "fruit".

E. Microorganisms

Table 38 is a listing of microorganisms reported in the literature to have shown some susceptibility to bromohydrocarbons.

Table 38. Microorganisms Known to Be Susceptible to Bromohydrocarbons

Agrobacterium tumefaciens; MeBr; 11344 (1962)

Bacillus anthracis; MeBr; 13475 (1952)

Bacillus subtilis; MeBr; 13118 (1966)

Coccidia; allyl bromide, 1,3-dibromopropene, 1,4-dibromo-2-butene; 12951 (1952)

Escherichia coli; MeBr; 13118 (1966)

Fanleaf-yellow Mosaic Virus; MeBr; 15506 (1971)

Pseudomonas angulata (Angular spot); $EtBr_2$; 10747 (1954)

Pseudomonas tabaci (Wildfire); $EtBr_2$; 10747 (1954)

Pseudomonas tomato; MeBr; 17785 (1954)

Rhizobium trifolii; MeBr; 17785 (1954)

Salmonella paratyphosus A, B; MeBr; 12939 (1952)

Salmonella typhosus; MeBr; 12939 (1952)

Shigella dysenteriae; MeBr; 12939 (1952)

Staphylococcus aureus; MeBr; 13118 (1966)

Tobacco Mosaic Virus; MeBr; 11299 (1962)

Vibrio cholerae; MeBr; 12939 (1952)

Xanthomonas vesicatoria; MeBr; 17785 (1954)

XI. CURRENT REGULATIONS

The following collection of foods and bromide residues permitted in them was obtained from the Federal Register through 1967; there had been only one change in the decade preceeding that year. No explanation for the different bromide ion tolerances from methyl bromide and ethylene dibromide treatment of the same food was found. When a food has been treated with both, the higher tolerance is used.

Table 39. Allowed Bromide Residues in Foods Treated with Bromohydrocarbons

Food	Methyl Bromide Tolerance[g]	Ethylene Dibromide Tolerance[g]	Other[f]
Alfalfa hay	50		
Apples	5		
Apricots	20		
Asparagus	100	10	
Avocados	75		
Barley	50	50, a	
Beans	50		
Beans, green	50		

Beans, lima	50	5	
Beans, snap	50		
Beets	30		
Broccoli		75	25
Cabbage	50		
Cantaloupe	20		
Carrots	30	75	
Cauliflower		10	25
Cereal grain,			
milled fractions	125	125	
Cheese,			
parmesan	325	325	
roquefort	325	325	
Cherries	20	25, b	
Cipollini bulbs	50		
Citrus citron	30		
Cocoa beans	50		
Copra	100		
Corn	50	50, a	
Corn, forage		50	
Cottonseed	200	25	
Cucumbers	30	30	
Dog food	400		
Dried apples	30		
apricots	30		

Dried dates	100		
eggs	C	C	
figs	150		
peaches	30		
pears	30		
Eggplant	20	50	60
Garlic	50		
Grain sorghum (milo)	50	50, a	
Grapefruit	30		
Grapes	20		
Hay, timothy	50		
Horseradish	30		
Jerusalem artichokes	30		
Kumquats	30		
Lemons	30	30	
Lettuce			
Limes	30		
Litchi fruit		10	
Mangoes	20		
Melons		75	
Melons, honeydew	20		
musk	20		40
water	20		

Nectarines	20		
Oat flour	d	d	
Oats	50	50, a	
Okra	30	50	
Onions	20		
Oranges	30		
Papayas	20		
Parsnips	30	75	
Peaches	20		
Peanuts		25	
Pears	5		
Peas,			
blackeyed	50		
with pods	50		
Peppers	30	30	25
Pimentos	30		
Pineapple	20	40	25
Plums	20	25, b	
Popcorn	240	50	
Potatoes,	75		
sweet	75	50	
Processed foods not already covered as of 6-15-66	125	125	

Processed grains for fermented malt beverages	e	e	
Processed herbs and spices	c	c	
Prunes	20		
Pumpkins	20		
Quince	5		
Radishes	30		
Raisins	50		
Rice	50	50, a	
Rutabagas	30		
Rye	50	50, a	
Salsify roots	30		
Soybeans	200		
Squash,			
summer	30	50	
winter	20		
zucchini	20		
Strawberries	30	5	25
Sugar-beets	30		
Tangelos	30		
Tangerines	30		
Tomatoes	20	50	40
Turnips	30		

Wheat	50	50, a
Yams	30	

a - no limit on organic bromide

b - total of organic and inorganic bromides

c - 400 from a mixture or from methyl bromide alone

d - 200 from a mixture

e - 125 from a mixture

f - a mixture of methyl bromide and propargyl bromide, in ppm of inorganic Br

g - in ppm of inorganic Br unless otherwise indicated

XII. STANDARDS

No information was found.

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Federal Register Regulations

No specific reference was made to these, but data were taken from all of them for the completion of the bromide residue tolerances given in Section XI.

Tolerances for residues of inorganic bromides (on agricultural commodities) from soil treatment with ethylene bromide. Fed. Regist. 21:768(Feb. 3, 1956) 10576

Tolerances for residues of inorganic bromides from fumigation with methyl bromides. Fed. Regist. 20:9822(Dec. 21, 1955) 10578

Exemption from requirement of tolerances for residues of carbon disulfide, carbon tetrachloride, ethylene dichloride, and organic bromide residues from ethylene dibromide; tolerances for inorganic bromide residues from ethylene dibromide. Fed. Regist. 21:5620(July 26, 1956) 10687

Food additives. Inorganic bromides. Fed. Regist. 31:8369-70(June 15, 1966) 10780

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Inorganic bromides. Tolerances resulting from fumigation with methyl bromide. Fed. Regist. 27:4623(May 16, 1962) 11345

Inorganic bromides; tolerance for residues. Fed. Regist. 26:12249(Dec. 22, 1961) 11401

Tolerances for residues of inorganic bromides resulting from fumigation with methyl bromide. Fed. Regist. 23:1365, 5465-66. (1958) 12156

Tolerances for residues of inorganic bromides from soil treatment with ethylene dibromide. Fed. Regist. 23:4002(June 7, 1958) 12157

Tolerances for residues of inorganic bromides in or on litchi fruit after fumigation with ethylene dibromide. Fed. Regist. 23:2966(May 2, 1958) 12393

Food additives. Fumigants for grain mill machinery. Fed. Regist. 28:6916 (July 6, 1963) 12606

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Inorganic bromides resulting from fumigation with methyl bromide. Fed. Regist. 32:7173(May 12, 1967)

Tolerances for residues of inorganic bromides resulting from soil treatment with ethylene dibromide. Fed. Regist. 22:4384-85(June 18, 1957) 13375

Tolerances for residues of total combined bromine in or on cherries and plums after fumigation with ethylene dibromide. Fed. Regist. 23:6553-54 (Aug. 23, 1958); 23:6665(Aug. 28, 1958) 13724

Inorganic bromides resulting from fumigation with methyl bromide; tolerances for residues. Fed. Regist. 30:2104(Feb. 16, 1965) 14184

Food additives. Inorganic bromide. Fed. Regist. 29:3394(Mar. 14, 1964) 14208

Inorganic bromides resulting from soil treatment with combinations of chloropicrin, methyl bromide, and propargyl bromide; tolerances for residues. Fed. Regist. 30:7385-86(June 4, 1965) 14724

Inorganic bromide resulting from soil treatment with ethylene dibromide; tolerance for residues. Fed. Regist. 30:14101(Nov. 9, 1965) 14954

Inorganic bromide; permitted residues from fumigation with methyl bromide. Fed. Regist. 25:8368-69(Sept. 1, 1960) 15192

Tolerance for residues of inorganic bromide. Fed. Regist. 25:8948-49 (Sept. 17, 1960) 15194

Food additives. Fumigants for grain-mill machinery. Fed. Regist. 29:4672 (Apr. 1, 1964) 14209

SUMMARY AND CONCLUSION AS TO DEGREE OF HAZARD

EDTA and its various Na, Ca, and other metal salts is being produced in fairly large quantities. The major uses do not degrade the organic portion of the compound and would appear to be ultimately resultant in its release to the environment. Ordinary sewage treatment does not degrade EDTA. Soil does not appear to retain EDTA, but can be "weathered" by it and also be subjected to minerals exchange.

There appears to be little danger to humans and domestic animals from oral ingestion because of the very low absorption from the gastrointestinal tract; only chickens show ability to absorb and metabolize EDTA. Plants readily abosrb EDTA into their roots, differ widely in ability to transport it, and show ability to metabolize it. Plants also vary widely in tolerance to the EDTA which they have absorbed. Insufficient information is available on toxicity to aquatic (including marine) life to arrive at any conclusion about the hazard of EDTA to these segments of the biosphere.

I. PHYSICAL PROPERTIES

Wendlandt (1960) ran differential thermal and thermogravimetric analyses on EDTA and two of its common salts, Na_2EDTA and Na_2CaEDTA, as obtained from various American suppliers. The results of his (values in this type of analysis are somewhat dependent upon the instrument and operational technique employed) DTA studies are given in Table 1. EDTA is seen to decompose at 250 or 265°, Na_2EDTA at 230-294°, and Na_2CaEDTA at 337-403°. In Table II are his TGA results, which indicate the manufacturer may over or underdry the hydrated salts. Wendlandt thought he had demonstrated the possibility that the calcium salt was a mixture of 1- and 3-hydrates, rather than the 2-3·H_2O previously suspected.

Bhat and Iyer (1967) ran TGA's on the following EDTA's: $BaH_2 \cdot 4H_2O$, $BiH \cdot H_2O$, $CaH_2 \cdot 2H_2O$, $CoH_2 \cdot 3H_2O$, $CuH_2 \cdot H_2O$, $DyH \cdot 2H_2O$, $NiH_2 \cdot H_2O$, and SbH. All of these decomposed before melting except Bi (292°), Cu (238°), and Sb (290°), the latter three decomposing just over their mp's. The Bi water of hydration was not liberated until after decomposition had set in, indicative of its being bound to the metal atom. The order of thermal stability found was Dy > Sb > Bi > Ni > Cu > Co > Ca, Ba; this order did not correlate with the stability constants or heats of formation in solution.

Koechel and Frank (1966) reported that: EDTA was soluble in alkalis, ethanol, ethyl ether, and chloroform, slightly soluble in water, and very slightly soluble in most other organics; Na_2EDTA was soluble in water to about one part in eleven (and gave a pH of 4.5 as a 0.1 M aqueous solution), but just barely soluble in organics; Na_2CaEDTA was

soluble in water on a one to one basis (and gave a pH of 7.5 as a 0.1 M aqueous solution). The sodium salts of EDTA exhibit the order of solubility in water: tetra > tri > di > mono. The dissociation constants of the four protons are: 1.0×10^{-2}, 0.2×10^{-2}, 6.3×10^{-7}, 5.0×10^{-11}, indicating that EDTA is a stronger acid than acetic.

Nomenclature of the EDTA's is complex, the term EDTA itself being indiscriminately applied to the tetra($-CO_2H$) and di($-CO_2H$)-di($-CO_2Na$) forms. Presented below is a collection of names compiled by the Chemical Abstracts Service of the American Chemical Society and published in Desktop Analysis Tool for the Common Data Base (1968).

Table I. Results of Thermal Analysis of EDTA and Its Derivatives
(Minimum thermogravimetric decomposition temperatures)

Compound	Transition	Temp., ° C.
EDTA (J. T. Baker)	EDTA → decomposition	250
EDTA (Sequestrene AA)	EDTA → decomposition	265
$Na_2EDTA.2H_2O$ (Sequestrene Na2)	$Na_2EDTA.2H_2O \rightarrow Na_2EDTA$	125
	$Na_2EDTA \rightarrow Na_2CO_3$	294
$Na_2EDTA\ 2H_2O$ (Eastman)	$Na_2EDTA\ 2H_2O \rightarrow Na_2EDTA$	114
	$Na_2EDTA \rightarrow Na_2CO_3$	256
$Na_2EDTA.2H_2O$ (J. T. Baker)	$Na_2EDTA.2H_2O \rightarrow Na_2EDTA$	110
	$Na_2EDTA \rightarrow Na_2CO_3$	255
$Na_2EDTA\ 2H_2O$ (J. T. Baker) (3° C. per min.)	$Na_2EDTA\ 2H_2O \rightarrow Na_2EDTA$	105
	$Na_2EDTA \rightarrow Na_2CO_3$	230
$Na_2CaEDTA\ 2\text{-}3H_2O$ (Sequestrene Na2Ca)	$Na_2CaEDTA\ xH_2O \rightarrow Na_2CaEDTA.3H_2O$	37
	$Na_2CaEDTA.3H_2O \rightarrow Na_2CaEDTA$	85
	$Na_2CaEDTA \rightarrow Na_2CO_3 + CaCO_3$	337
$Na_2CaEDTA.2\text{-}3H_2O$ (Sequestrene Na2Ca) (3° C. per min.)	$Na_2CaEDTA\ xH_2O \rightarrow Na_2CaEDTA.3H_2O$	45
	$Na_2CaEDTA\ 3H_2O \rightarrow Na_2CaEDTA\ 1H_2O$	63
	$Na_2CaEDTA\ 1H_2O \rightarrow Na_2CaEDTA$	123
Endotherm Peak Maxima		
EDTA (J. T. Baker)	EDTA → decomposition	255
EDTA (Sequestrene AA)	EDTA → decomposition	257
$Na_2EDTA.2H_2O$ (J. T. Baker)	$Na_2EDTA\ 2H_2O \rightarrow Na_2EDTA$	195
	$Na_2EDTA \rightarrow$ decomposition	255
$Na_2EDTA.2H_2O$ (Eastman)	$Na_2EDTA.2H_2O \rightarrow Na_2EDTA$	185
	$Na_2EDTA \rightarrow$ decomposition	253
$Na_2EDTA.2H_2O$ (Sequestrene Na2)	$Na_2EDTA\ 2H_2O \rightarrow Na_2EDTA$	212
	$Na_2EDTA \rightarrow$ decomposition	255
$Na_2CaEDTA.2\text{-}3H_2O$ (Sequestrene Na2Ca)	$Na_2CaEDTA\ xH_2O \rightarrow Na_2CaEDTA\ 3H_2O$	107
	$Na_2CaEDTA\ 3H_2O \rightarrow Na_2CaEDTA\ 1H_2O$	168
	$Na_2CaEDTA\ 1H_2O \rightarrow Na_2CaEDTA$	190
	$NaCaEDTA \rightarrow$ decomposition	318 403

Table II. Weight Loss Data for EDTA and Its Derivatives

Compound	Water, % Experimental	Water, % Theoretical
$Na_2EDTA \cdot 2H_2O$ (J. T. Baker)	10.5	9.68
	10.2	
	9.7	
	10.1	
$Na_2EDTA \cdot 2H_2O$ (Eastman)	10.1	9.68
	10.0	
$Na_2EDTA \cdot 2H_2O$ (Sequestrene Na2)	9.3	9.68
	9.5	
$Na_2CaEDTA \cdot 2-3H_2O$ (Sequestrene Na2Ca)	1.72 (residual water)	
	1.93	
	1.81	
	11.7 (3-hydrate)	12.62
	11.8	
	12.6	
	4.34 (1-hydrate)	4.59
	4.29	
	5.00	

Table IIa. Synonyms for EDTA and its Salts

$N_2O_8C_{10}H_{12} \cdot 4Na$

Acetic acid, (ethylenedinitrilo)tetra-, tetrasodium salt
Aquamollin MERCK
Calsol MERCK
Celon E IECMTN
Celon H IECMTN
Celon IS IECMTN
Cheelox BF
Cheelox PR-33
Complexone MERCK
Conigon BC IECMTN
Distol B MERCK
Distol
Edathanil tetrasodium ADI
EDTA, sodium salt CARF
EDTA tetrasodium salt MERCK,IECMTN
Endrate tetrasodium MERCK
Ethylenebis[iminodiacetic acid] tetrasodium salt MERCK
N,N'-Ethylenediaminediacetic acid tetrasodium salt IECMTN
Ethylenediaminetetraacetic acid, tetrasodium salt ADI,MERCK,CFR
(Ethylenedinitrilo)tetraacetic acid tetrasodium salt MERCK
Irgalon MERCK
Kalex IECMTN,MERCK
Komplexon MERCK
Metaquest C IECMTN
Nervanaid B MERCK
Nullapon MERCK
Nullapon BF-12 IECMTN
Nullapon BF-78 IECMTN
Nullapon BFC Conc IECMTN
Nullapon BFC Conc Beads IECMTN
Nullapon BFC liquid IECMTN
Perma Kleet 50 crystals IECMTN
Questex MERCK
Questex
Sequestrene MERCK
Sequestrene 30A IECMTN
Sequestrene
Sodium edetate USAN
Sodium EDTA
Sodium ethylenediaminetetraacetate
Sodium ethylenediaminetetraacetic acid
Sodium salt of ethylenediaminetetraacetic acid IECMTN
Syntex 1Ca MERCK

Tetracemin MERCK
Tetrasodium EDTA IECMTN
Tetrasodium ethylenediaminetetraacetate IECMTN,CFR,USAN
Tetrasodium ethylenediaminetetracetate
Tetrasodium (ethylenedinitrilo)tetraacetate USAN
Tetrasodium salt EDTA IECMTN
Tetrasodium salt of EDTA IECMTN
Tetrasodium salt of ethylenediaminetetracetic acid IECMTN,VBB
Tetrine MERCK,IECMTN
Trilon B MERCK,IECMTN
TST
Tyclarosol MERCK
Versene 67 IECMTN
Versene 100 IECMTN
Versene MERCK
Versene Beads IECMTN
Versene FE 3 ADI
Versene Powder IECMTN
Warkeelate S-42 IECMTN
Warkeelate PS-42 IECMTN
Warkeelate PS-43 IECMTN
Warkeelate PS-47 IECMTN

$N_2O_8C_{10}H_{16}$
Acetic acid, (ethylenedinitrilo)tetra-
Celon A IECMTN
Celon ATH IECMTN
Complexon II
3,6-Diazaoctanedioic acid, 3,6-bis(carboxymethyl)-
Edathamil IECMTN
EDETIC ADI
Edetic acid INN,INN-A
EDTA CDF
EDTA IECMTN
EDTA acid IECMTN
Endrate
Ethylenediaminetetraacetate IECMTN
Ethylenediaminetetraacetic acid CFR,IECMTN
Ethylenediamine-N,N,N',N'-tetraacetic acid
(Ethylenedinitrilo)tetraacetic acid
Metaquest A IECMTN
Nervanaid B acid IECMTN
Nullapon B acid IECMTN
Nullapon BF acid IECMTN
Perma Kleer 50 acid IECMTN
Sequestrene AA IECMTN
Sequestric acid IECMTN
Sequestrol IECMTN
Tetrine Acid
Trilon B, Trilon BW
Trilon BW
Versene
Versene acid IECMTN
Warkeelate Acid IECMTN

$N_2O_8C_{10}H_{16}\cdot 3Na$
Acetic acid, (ethylenedinitrilo)tetra-, trisodium salt
EDTA trisodium salt IECMTN
Ethylenediamineacetic acid trisodium salt MERCK
Perma Kleer 50, trisodium salt IECMTN
Sequestrene trisodium
Sequestrene trisodium salt MERCK
Trilon AO IECMTN
Trisodium edetate USAN
Trisodium EDTA IECMTN
Trisodium hydrogen ethylenediaminetetraacetate USAN
Trisodium hydrogen (ethylenedinitrilo)tetraacetate USAN
Trisodium versenate CARF
Versene 9 IECMTN,MERCK

$N_2O_8C_{10}H_{16}\cdot 2Na$

Acetic acid, (ethylenedinitrilo)tetra-, disodium salt
Complexon III IECMTN
Disodium diacid ethylenediaminetetraacetate
Disodium dihydrogen ethylenediaminetetraacetate FCC
Disodium edetate USAN,USP,USP-A
Disodium EDTA IECMTN,FCC,CFR,MDE,ADI
Disodium ethylenediaminetetraacetate FCC,USP,VBB,CFR
Disodium ethylenediaminetetraacetic acid MDE
Disodium (ethylenedinitrilo)tetraacetate USP
Disodium (ethylenedinitrilo)tetraacetic acid
Disodium salt of EDTA IECMTN
Disodium sequestrene CARF
Disodium versene
Edathamil disodium ADI,CARF
Edetate Disodium ADI
EDTA
EDTA, disodium salt
EDTA disodium CARF
Endrate disodium MDE,ADI,CARF
Ethylenediaminetetraacetate, disodium salt CARF
Ethylenediaminetetraacetic acid, disodium salt ADI
Metaquest B IECMTN
Perma Kleer 50 crystals disodium salt IECMTN
Sequestrene sodium 2 UD
Triplex III IECMTN

$N_2O_8C_{10}H_{16}\cdot 2OH_2\cdot 2Na$

Acetic acid, (ethylenedinitrilo)tetra-, disodium salt, dihydrate
Disodium dihydrogen ethylenediaminetetraacetate dihydrate FCC
Disodium EDTA dihydrate IECMTN
Disodium ethylenediaminetetraacetate dihydrate FCC,IECMTN
EDTA disodium dihydrate CARF
Sequestrene NA2 IECMTN

$N_2O_8CaC_{10}H_{12}\cdot Ca$

Acetic acid, (ethylenedinitrilo)tetra-, calcium salt (1:1)
Calcium versenate CARF
EDTA Calcium salt CARF

$N_2O_8C_{10}H_{16}\cdot 2Ca$

Acetic acid, (ethylenedinitrilo)tetra-, dicalcium salt
Ca-EDTA
Calcium EDTA
Calcium ethylenediamine tetraacetate CARF
Calcium tetracemin
Dicalcium EDTA
EDTA, calcium salt CARF

$N_2O_8CaC_{10}H_{12}\cdot 2Na$

Acetic acid, (ethylenedinitrilo)tetra-, calcium disodium salt
Calciate(2-), [(ethylenedinitrolo)tetraacetato]-, disodium
Calcium EDTA
Calcium ethylenediaminetetraacetate
Di-sodium calcium EDTA
Edathemil calcium disodium UD

$N_2O_8CaC_{10}H_{14}\cdot 2Na$

Antalin MERCK
Calciate(2-), [(ethylenedinitrilo)tetraacetato]-, disodium
Calcium disodium edetate USP,CTCP
Calcium disodium ethylenediaminetetraacetate USP
Calcium disodium (ethylenedinitrilo)tetraacetate MERCK,USP,CTCP,FCC
Calcium Disodium Versenate MERCK
Edathamil calcium disodium MERCK
Ethylenediaminetetraacetic acid, calcium disodium chelate MERCK
Mosatil MERCK

$N_2O_8C_{10}H_{16}\cdot 2OH_2\cdot Ca\cdot 2Na$

Calcium disodium edetate dihydrate FCC
Calcium disodium EDTA dihydrate FCC
Calcium disodium ethylenediaminetetraacetate dihydrate FCC
Calcium disodium (ethylenedinitrilo)tetraacetate dihydrate FCC
Sodium [(ethylenedinitrilo)tetraacetato]calciate, $Na_2[Ca(C_{10}H_{12}N_2O_8)]$, dihydrate

$N_2O_8CaC_{10}H_{14}\cdot 2Na\cdot xOH_2$

Antallin CDF
Calciate(2-), [(ethylenedinitrilo)tetraacetato]-, disodium, hydrate
Calcium disodium edetate USAN,USAN-A
Calcium disodium (ethylenedinitrilo)tetraacetate hydrate USAN
Calcium disodium versenate USAN
Edathamil USAN

$N_2O_8C_{10}H_{14}\cdot 2Na\cdot Zn$

Disodium zinc EDTA IECMTN
Sequestrene NA2ZN IECMTN
Sodium zinc ethylenediaminetetraacetic acid
Zincate(2-), [(ethylenedinitrilo)tetraacetato]-, disodium

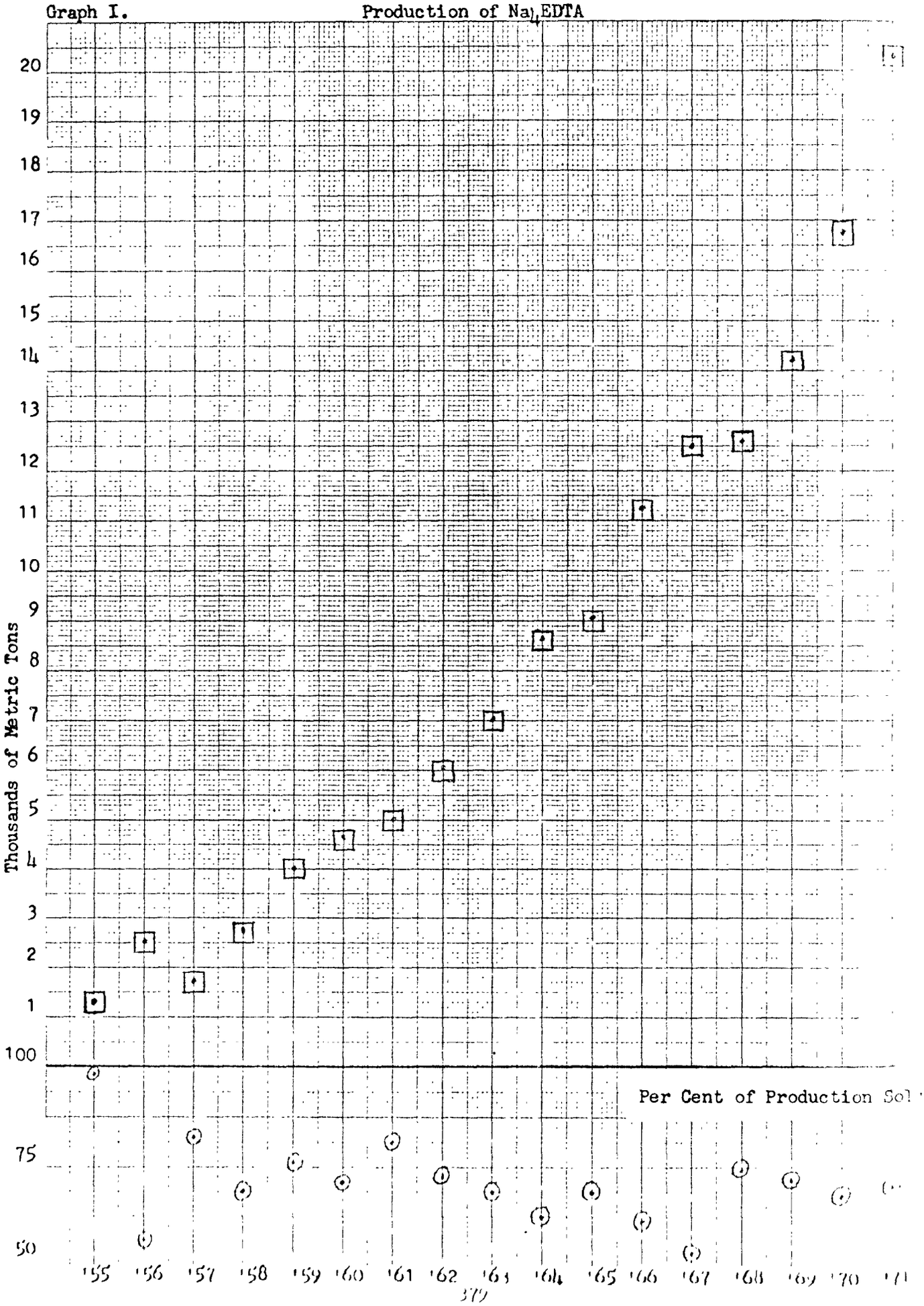
$N_2O_8C_{10}H_{14}\cdot xFe$

Acetic acid, (ethylenedinitrilo)tetra-, iron salt
Dihydrogen ferrous EDTA IECMTN
EDTA iron(II)
Sequestrene H2FE IECMTN

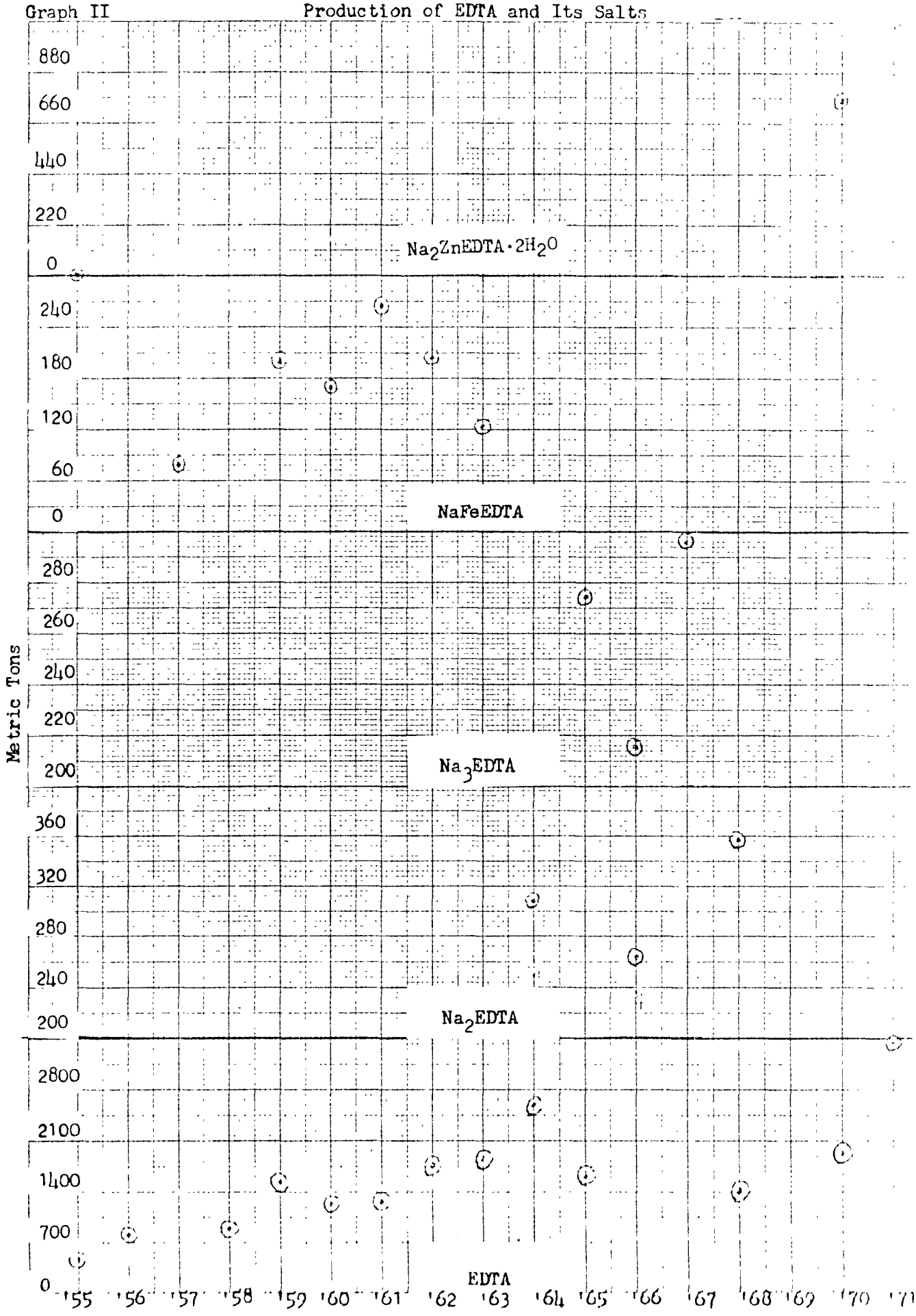
$N_2O_8C_{10}H_{14}\cdot xFe\cdot xNa$

Acetic acid, (ethylenedinitrilo)tetra-, iron complex, sodium salt
Edta iron sodium CARF

Graph I. Production of Na_4EDTA



Graph II Production of EDTA and Its Salts



A number of the formulas given are incorrect in that they retain the H's displaced by the metal ions.

II. PRODUCTION

Production figures for the tetrasodium salt of EDTA are available for the years 1955-1971 and are presented in Graph I; this salt is the material from which EDTA itself and the other salts are prepared. Available statistics for EDTA and other salts are shown in Graph II. None of these figures should be taken literally because it is only the tetrasodium salt which is being "made." It, at least, shows a clear upward trend, seemingly at a geometric rather than the earlier algebraic increase; the percent actually sold is moderately steady at about 70. Prior to 1968 about 50-55% of EDTA production was sold, but then the figure dropped sharply to about 30%. For the NaFeEDTA, there was a 50-50 chance of reported sales exceeding reported production. The 1971 U.S. Tariff Commission Report indicated the following companies to be producers of the noted EDTA's: Ciba-Geigy Corp.—Na_4-, Na_3-, Na_2-, EDTA, K_4, NaFe-, Mn-, Na_2Cu-, Na_2Ca-, Na_2Zn-; Crest Chemical Corp.—Na_4-; Dan River, Inc.—Na_4-; Dow Chemical Co.—Na_4-, Na_2, EDTA, $(NH_4)_4$-, $(NH_4)_2$-, Na_2Ca-, Na_2Zn-; Eastman Kodak Co.—Na_2-; W.R. Grace & Co.—Na_4-, Na_3-, Na_2-, EDTA, K_4-, NaFe-, Mn-, Na_2Cu-, Na_2Zn-; Hart Products Corp.—Na_4-; Millmaster Onyx Corp.—Na_4-.

III. USES

The following table (III) was presented in Chemical Economics Handbook (1967), but it was not exclusive to EDTA.

CHEMICAL ECONOMICS HANDBOOK, Stanford Research Institute, Menlo Park, California, p. 512.5020R

Table III. Estimated Markets for Aminopolycarboxylic Acid Chelating Agents, 1965

Textiles	30%
Soap and Cleaning Compounds	20
Water Treatment	15
Miscellaneous Chemical Processing	15
Agriculture	5
Rubber Processing	5
Metal Cleaning and Electroplating	5
All Other (Largely Pulp and Paper Processing	<10

Source: CEH estimate based on communication with industry

CEH elaborated on these uses as follows: textiles - improvement of dyeing evenness, extension of life of alkaline bleaches, water softener in cleaning operations; soap and cleaning compounds - water softener, foam stabilizer, builder; water treatment - prevention of scale in boiler water, scavenging of limey deposits; miscellaneous chemical - improvement in product quality and yield, catalyst recovery in petroleum products, mineral flotation separation adjunct, rare earth separation, pre-ion exchange treatment; agriculture - correction of mineral deficiencies, water softener for spraying operations; rubber - copolymerization activator, metal scavenger; metal cleaning - "rust" and lime remover, metal scavenger, etchant. Water treatment was the anticipated area of fastest growth.

EDTA has some important, but probably only small quantity, use in analytical chemistry as a titrant for metals.

Considerable amounts (relative to human contact) may be used in foodstuffs, the disodium and the calcium disodium EDTA being the only salts allowed. Table IV, adapted from the 1972 edition of the Handbook of Food Additives, lists the allowable amounts and the specific foods permitted to contain them; there have been no changes in the allowable amounts since 1960.

Table IV. Regulatory Status of Direct Food Additives

$CaNa_2EDTA$ 121.1017	Material FDA Regulation Limitations	Na_2EDTA 121.1056
Alone, as food additive 33 ppm max in canned carbonated soft drinks 110 ppm max in canned white potatoes 340 ppm max in canned cooked clams 275 ppm max in canned cooked crabmeat 25 ppm max in distilled alcoholic beverages 75 ppm max in non-standardized dressings 310 ppm max in canned, cooked, dried lima beans 25 ppm max in fermented malt beverages 75 ppm max in French dressing 75 ppm max in mayonnaise 200 ppm max in canned, cooked mushrooms 75 ppm max in oleomargarine 100 ppm max in pecan pie filling 220 ppm max in pickled cabbage 220 ppm max in pickled cucumbers 100 ppm max in potato salad		1. Alone, as food additive 150 ppm max in aqueous multivitamin preparations, with iron salts as stabilizer for vitamin B_{12} 145 ppm max in canned, black eyed peas 165 ppm max in canned cooked chick peas 165 ppm max in canned kidney beans 500 ppm max in canned strawberry pie filling 36 ppm max in coated sausage 75 ppm max in non-standardized dressing 315 ppm max in dried banana component of ready-to eat cereal products 75 ppm max in French dressing 100 ppm max in frozen white potatoes, including cut potatoes 50 ppm max in gefilte fish balls or patties, including liquid packing medium, to inhibit discoloration 75 ppm max in mayonnaise

800 ppm max in processed, dry pinto beans
75 ppm max in salad dressings
100 ppm max in sandwich spreads
75 ppm max in sauces
250 ppm max in canned, cooked shrimp
60 ppm max in spice extractives in soluble carriers
100 ppm max in artificially flavored lemon and orange spreads

In combination with disodium EDTA as food additive:
75 ppm max in non standardized dressings
75 ppm max in French dressing
75 ppm max in mayonnaise
75 ppm max in salad dressing
100 ppm max in sandwich spread
75 ppm max in sauces

Product specifications apply

75 ppm max in salad dressing
100 ppm max in sandwich spread
75 ppm max in sauces

2. In combination with calcium disodium EDTA as food additive:
75 ppm max in non standardized dressing
75 ppm max in French dressing
75 ppm max in mayonnaise
1000 ppm max (dry-weight basis) in nonnutritive sweeteners

T.E. Furia, in Chapter 6 of this Handbook, discussed the purposes of using EDTA's in various foods. Fats and oils, and foods containing them, were protected by a synergistic combination of EDTA and antioxidant (BHA, BHT, ascorbic acid, etc.). Aqueous vitamin preparations, especially vitamin C, or oil soluble vitamins such as A, D, E, and K were stabilized by EDTA's (in conjunction with antioxidants for the oil solubles). Processed fruits and vegetables suffered less color changes and alterations in flavor or texture. Fish and shellfish had improved color stability and less tendency to form the glass-like crystals called struvite. Wine, cider, and vinegar showed much less tendency to form precipitates. Milk was kept from developing off flavors resultant from copper contamination. Various benefits accrued to the beer and sausage manufacturing processes.

IV. CURRENT PRACTICE

No information concerning handling and transportation regulations or disposal methods was found.

V. ENVIRONMENTAL CONTAMINATION

No information concerning environmental occurrence was found.

VI. MONITORING AND ANALYSIS

A variety of chromatographic, spectrophotometric, and titrimetric procedures has been developed which bypass the presence of the usual cations associated with EDTA systems; the cations may be detected and quantitized by standard methods in inorganic analysis.

Heinerth (1968) detected EDTA in detergents by thin layer chromatography after preliminary extraction and removal of interfering salts. The medium was Kieselgel G.

Yamagata *et al* (1969) developed a thin layer chromatographic technique especially useful for separating the EDTA used in foods from amino acids, particularly aspartic. Their medium was the cellulose powder Avicel SF, and the solvent system n-butanol/acetic acid/water (1/2/2 by volume). The spot was detected by spraying with acetic acid, cobaltous chloride, and hydrogen peroxide. The Rf for EDTA was about 40% greater than that for aspartic acid. The limit of detection was 1.5 µg.

Mihara *et al* (1970) analyzed food for EDTA by gas liquid chromatography after conversion to the methyl ester by simply refluxing in acidic methanol (claiming that diazomethane or boron trifluoride-methanol esterification was unsuitable). Detection limits were 8.4 ng and 12 ng on 4-mm X 1.08-m 5% QF-1 on Gas-Chrom Q at 175° or 3-mm X 1.5-m OV-1 on Gas-Chrom Q at 185° columns, respectively.

Rudling (1972) analyzed for EDTA in water or sewage in the presence of nitrilotriacetic acid (NTA) and diethylene-triaminepentaacetic acid (DTPA) by conversion to the methyl ester and gas-liquid chromatography. The esterifying agent was boron trifluoride in aqueous methanol. The chromatographic system consisted of a 100 X 0.2 cm i.d. glass column packed with 5% (w/w) OV-17 on 100/120 mesh Aeropak, helium carrier gas, and a flame ionization detector. The EDTA ester eluted at 12 minutes into a 10°C/min. programmed rise from 150°C. The minimal concentration detectable was 10 µg/l (about 10 ppb). A solution containing 0.2 mg/l of EDTA was analyzed without interference from 2 mg/l concentrations of Cd(II), Cu(II), Fe(III), Ni(II), or Zn(II).

Menis *et al* (1956) measured EDTA by forming a Cu(II) complex and measuring the absorbance at 250 nm. Good results were obtained at concentrations down to 0.1 g/l, not quite as good in the 0.025-0.1 g/l range. At the 0.05 g/l level of EDTA, interferences came from Cr(VI), Ni(II), and Co(II) (the latter only when present in amounts over 10% of the EDTA).

Vogel and Deshusses (1962) determined EDTA in wine by forming a complex with Co(II), then oxidizing with peroxide to the Co(III) complex, and measuring the absorbance at 530 nm. A minimum of 2 ppm EDTA was detected.

Stahlavska and Malat (1965 and 1965) analyzed pharmaceuticals for EDTA by using it to displace various heavy metals from phenolic chelates, and measuring the remaining absorbance. Concentrations of EDTA as low as 0.7 µg/ml were detectable.

Suk and Smetanova (1965) added excess Bi(III) to an EDTA solution, adjusted the pH to 2.0, added bromopyrogallol red, and measured the

absorbance of the Bi-catechol complex at 635 nm.

Mottola and Freiser (1967) demonstrated the feasibility of measuring sub-micromolar quantities of EDTA by the inhibiting effect it has on the catalysis by Mn(II) of the oxidation of malachite green by periodate. However, many of the non-alkaline earth metals and also other polyacetic acid complexants interfered severely.

Kross (1968) patented a method for determining EDTA in meat products. The EDTA was complexed with Ni(II), the complex destroyed by oxidation, and the liberated Ni(II) complexed with dimethylglyoxime for spectrophotometric measurement at 430 nm.

Ishihara (1968) added excess acidic zirconium solution to an EDTA sample, then added xylenol orange and measured the absorbance of its complex with the non-chelated Zr at 530 nm. Various common cations and anions interfered, otherwise the minimum detectable amount of EDTA being 50 µg.

Shimokawa and Horibe (1968) determined EDTA in food by measuring the absorbance of the cobalt complex after removing interfering amino acids by passing the dissolved sample over a column of the anion-exchange resin Amberlite IR-45 at pH 2.1. The limit of detection was 0.4 mg.

Saito *et al* (1968) used the cobalt/peroxide method (Vogel and Deshusses, above) for EDTA in sake. They reported a useful range of 5-600 ppm, and cautioned that the pH of the final solution must be 3.0 to prevent interference from any amino acids. Common inorganic and organic acids and salts at concentrations below 0.1% did not interfere.

Bruno *et al* (1969) used this same method for EDTA in fruit juice but measured the absorbance at the slightly higher wavelength of 535 nm. Their errors on spiked samples were ± 5%.

Bhattacharyya and Kundu (1971) determined concentrations of EDTA in the μM region by adding excess Fe(III) and measuring the absorbance of the non-chelated and chelated iron at 305 and 258 nm, respectively. Most common cations did not interfere.

Krówczyński and Banaszek (1958) measured EDTA, Na_2EDTA, $Na_2CaEDTA$, and Ca_2EDTA in pharmaceutical preparations by titration with Fe(III) using sulfosalicylic acid indicator.

Hennart and Merlin (1958) checked on the purity of $Na_2CaEDTA$ by separately determining the Ca and Ca plus Na. A sample was ignited to the mixed carbonates and divided in two. One portion was dissolved in acid, adjusted to pH10, and then titrated with EDTA to determine Ca. The other portion was dissolved in perchloric acid/propanoic acid and the excess perchloric titrated with pyridine/propanoic acid with malachite green to determine Ca plus Na.

Clinckemaille (1968) determined EDTA in detergents by titration with Cu(II) and the indicator commonly written as PAN; one percent of any nitrilotriacetic acid present would titrate under the conditions used and would have to be determined separately for accurate work.

Heinerth (1968) also used Cu(II) to titrate EDTA in detergents, at pH 4 and 60°C, but with the indicator polyacrylonitrile; under these conditions hydroxy-EDTA also titrated.

Huber and Tallant (1968) titrated EDTA solutions with Pb(II) using constant current potentiometry as an endpoint indicator. Concentrations at the sub-mM level were determined with good accuracy. The common anions and Ca(II) did not interfere; phosphate ions at the mM level interfered with the normal way of running the analysis but could be counteracted by plotting the experimental data; no Mn(II) could be tolerated.

Treffler (1968) discussed an extraction/titration technique for determining EDTA in powdered alkaline cleaning compositions.

Vanderdeelen and Van den Hende (1968) added excess bismuth ion to an EDTA solution, and then titrated the uncomplexed Bi with standardized EDTA using pyrocatechol violet indicator. Under the conditions used, only Hg(II) and oxalate ions interfered.

Blijenberg and Leijnse (1969) titrated EDTA in blood or urine with Cu(II) using the indicator pyridylazonaphthol and a visual or colorimetric endpoint. Large excesses of Ca(II), Mg(II), phosphate, or citrate were non-interferants.

Groninger and Brandt (1969) determined EDTA in fish and shellfish by titration with Th(IV).

Reuge (1971) determined CaEDTA in protein solutions by titrating with Zn(II) after precipitating the Ca with oxalate.

Milwidsky (1971) reported a method for determining EDTA in the presence of detergent phosphates. A sample containing at least 0.1 g EDTA was adjusted to pH 2.5, Zn(II) added, and the pH readjusted potentiometrically with NaOH to 2.5. The amount of NaOH corresponded to the amount of EDTA. Any nitrilotriacetic acid present interfered.

Titrimetric procedures for determining the purity of calcium disodium EDTA and disodium EDTA were described in Food Chemicals Codex (1972), pages 128 and 259, respectively. The former was titrated with thorium against xylenol orange. The disodium was converted to the calcium complex, then titrated with NaOH against hydroxy-naphthol blue.

VII. CHEMICAL REACTIVITY

A. Environmental and use associated reactions

Most of the reactions EDTA and its salts undergo are simple

complexations of polyvalent metal ions after displacing sodium atoms or another metal from the EDTA. In general the complexes are formed more readily at high pH than at low because the H's are more apt to be ionized at the former; preformed complexes of tri- and tetravalent metals are stable at pH's $\leq$ 1.

Deleted because of copyright clearance

Cheronis and Schatz (1958) claimed that EDTA catalytically degraded basalt, limestone, or shale rocks. They showed that an aqueous EDTA solution covering the rocks gradually increased in pH and acquired coloration, whereas omitting the EDTA resulted in no such changes.

B. Aspects with biological implications

Hashimoto (1966) demonstrated that EDTA interfered with the ability

of volcanic ash or red soils to fix phosphorus, but enhanced this ability in a calcareous whitish soil.

Ikehata *et al* (1967) studied the effect of EDTA on the formation of flocculated $Al(OH)_3$ in water treatment processes.

Anghileri (1968) demonstrated that EDTA could bind to serum albumin and possibly interfere with the albumin's metal-binding ability.

Singh (1971) thought that some light on the toxic side-reactions from therapeutic EDTA dosing might have been shed by showing that, in a non-chelating fashion, EDTA could dissociate both ionically and non-ionically linked complexes of polymerized DNA.

VIII. BIOLOGY

A. Metabolism

1. Absorption

In a study on normal human adult males Foreman and Trujillo (1954) found that only about five percent of an oral dose of $CaNa_2EDTA$ was absorbed, and that almost none of a skin applied dose was absorbed. Wallace *et al* (1955) discussed the absorption from soil by various plants (see VIII. 3 and 4, and X. D. for other findings of this study). Foreman (1959) reported that rats didn't absorb much EDTA from an oral dose, but did absorb more if they had been fasted beforehand. Spencer (1960) studied the absorption of oral doses of CaEDTA or Na_2EDTA in man at the rate of 6 gm/day for 6 days. Some of the subjects absorbed a little of the dose, others none at all. Wallace and Mueller (1966) applied FeEDTA (both Fe and C isotope labelled) to an alga, but could not determine if only the Fe was absorbed, or the whole complex absorbed and EDTA portion immediately eliminated. Kealy *et al* (1969) indicated that chicks fed Na_4EDTA absorbed more than half.

Wynn *et al* (1970) fed male rats diets containing up to 10% by weight of Na_2EDTA for 13 weeks. Some of the EDTA was absorbed, apparently as the CaEDTA form, but never exceeded a serum level of 1 mg/100 ml.

2. Excretion

Foreman and Trujillo (1954) subjected normal adult male humans to intravenous, intramuscular, oral, or skin doses of $CaNa_2EDTA$ at levels of 2002.2 mg, 1002.2 mg, 1.5 mg, and 1002 mg, respectively. Within the accuracy of their analytical method all of the i.v. and i.m. dose was recovered in the urine in a 24 hour period. Of the oral dose a minimum of 91% was recovered within three days in the feces and urine at a 23/1 ratio. At most 0.001% of the skin dose appeared in the urine. The half times for blood clearance after i.v. or i.m. dosing were 65 and 90 minutes, respectively; there was no detectable EDTA in the blood after oral or skin dosing. There was no indication of the presence of metabolites in the urine. The renal clearance value of 680 ml/min. after i.v. dosing indicated that glomerular filtration and tubular excretion both played parts in the clearance.

To study the stability of EDTA in plants Wallace *et al* (1955) grew orange seedlings for 60 and 110 day periods in soil containing EDTA bearing isotopically labelled nitrogen, then water-extracted the leaves and chromatographed the extract over cation and anion exchange resins. EDTA itself is not retained on cation resins, but a considerable fraction of the radioactivity was found on the cation resin after both periods of growth, indicative of degradation of the EDTA.

Foreman (1959) reported that rats given EDTA parenterally excreted 97.5% within six hours, with a blood turnover time of 57 minutes after

i.v. dosing. Clearance from the blood occurred only through the kidney. The remaining 2.5% was released slowly, possibly having been bound to iron strongly fixed to something. Tubular secretion and glomerular filtration were involved. These results paralleled the author's work with humans (above).

Spencer (1960) reported that essentially all of an EDTA dose given to three human subjects was eliminated in the urine in a 24 hour period, in agreement with the Foreman study (above), but, perhaps, experimentally more valid.

Darwish and Kratzer gave 7.4 μm doses of C-14 labelled EDTA orally to laying hens which had been colostomized. The serum plasma EDTA level peaked at about 0.1% of the dose at about one hour, then dropped rapidly and leveled to about 1/4 this level, where it remained for almost two days. Carbon-14 in the respired air peaked at 7 and 28 hours in one bird, at 7 and ? hours at a higher level in the other bird (experiment with this bird terminated at 42 hours); there was still activity in the first bird at 110 hours. Urinary C-14 peaked at about 11 hours in the 42-hour bird, about 1/3 as uric acid. After 144 hours recovery of the dose from one bird amounted to 4% in the expired air, 9% in the urine, 52% in the feces, and 1% in a G.I. tract washing.

Havlicek *et al* (1968) gave adult rats intraperitoneal injections of EDTA (Ca and Y cations, 1 and 100 μM doses, neither being a factor in the results) labeled with C-14 in the $-C^*O_2H$, $-C^*H_2CO_2H$, $-C^*H_2N-(CH_2CO_2H)_2$ positions. After 24 hours about 1.2% of the C-14 in the first two of these, but only 0.05% of the C-14 in the last, showed up in the expired air. In the same period 95 ± 6% of the dose showed up in the urine, and about 1/4% in the feces (C-14 in the C^*O_2H). There

was some evidence for most of the decomposition occurring in the kidneys.

3. Transport

Foreman and Trujillo (1954) determined that one hour after an i.v. dose of $CaNa_2EDTA$ in a male human adult the level of EDTA in the spinal fluid was only 1/20 that in the blood plasma, indicative of very slow transport across the blood-spinal fluid barrier.

Wallace *et al* (1955) grew bean plants in nutrient solutions containing varying amounts of Na_2EDTA. Table VI indicates that there wasn't any linear relationship between the concentrations of EDTA in the nutrient, roots, or plant top. The plants seemed to be able to absorb the EDTA faster than they could transport it.

Tanton and Crowdy (1971) reviewed the use of PbEDTA as an agent for the study of transport in plants.

TABLE VI—YIELD, EDTA AND MINERAL CONTENTS AS PER CENT OF DRY WEIGHT OF BEAN PLANTS GROWN 14 DAYS IN NUTRIENT SOLUTIONS CONTAINING VARIOUS CONCENTRATIONS OF Na_2EDTA BUT WITH CONSTANT LEVELS OF MICRONUTRIENTS.

Na-EDTA in nutrient solution (ppm)	Dry weight (g/plant)	Water soluble EDTA in plant* (%)		Fe (ppm)		Mn (ppm)		Zn (ppm)		EDTA in comparable citrus leaves* (%)
		Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	
0	6.0	T	T	61	6202	40	85	73	241	T
10	6.9	T	0.10	72	1812	39	107	75	238	0.06
50	5.7	0.01	0.12	106	1230	58	91	55	161	0.08
100	5.7	0.12	0.12	113	1780	47	52	55	141	0.08
500	4.3	0.13	0.26	76	1710	21	18	58	137	0.08
1000	2.3	0.49	1.05	137	1200	35	29	81	115	0.09
2000	1.1	0.61	2.90	203	1800	27	25	112	152	0.11
F value	11.8**	17.2**	7.6**	3.3	23.5**	2.6	7.0*	7.8**	5.9*	
LSD (.05)	1.9	0.11	0.54	N.S.	1542	N.S.	31	41	65	-

*Some plant materials give positive test with reagents even though EDTA is not present.
'T' means trace.

4. Distribution

Foreman and Trujillo (1954) calculated that shortly after i.v. injection, EDTA left the blood stream and permeated the body's entire water supply, exclusive of spinal fluid and red blood cells.

Wallace *et al* (1955) measured the distribution of N-15 labelled EDTA in orange cuttings; their results are given in Table VII.

TABLE VII.-Labiled N Coming from EDTA, Calculated Iron Equivalents, and Iron Contents of Orange Cuttings Supplied N^{15}-Labelled NaFeEDTA for Four Days.[a]

Plant part	Labeled N (%)	Iron in treated plants (ppm)	Iron in nontreated plants (ppm)	Iron difference (ppm)	Calculated iron equivalent of EDTA in treated plants[b] (ppm)
Leaf margins	0.00188	116	81	35	37.6
Inner part of leaf	0.00262	116	88	28	52.4
Leaf vein	0.00122	126	102	24	24.4
Petioles	0.00100	114	83	31	20.0
Bark	0.00164	120	65	55	32.8
Wood	0.00038	62	44	18	7.6
Callus	0.00148	205	148	57	29.6
Root bark	0.00549	1404	568	868	109.8
Root wood	0.00084	138	92	46	16.8
Fine roots	0.00580	1772	1255	517	116.0

[a]On dry weight basis.
[b]From the labeled N content. The labeled N was derived from EDTA.

Foreman (1959) reported that no organ of the rat concentrated $CaNa_2EDTA$ to any extent. The EDTA was distributed over a volume a bit greater than the extracellular space.

Matsuda (1968) confirmed, with tomato plants, Wallace's observation that EDTA concentration was greater in roots than plant tops.

Weber (1969) gave adult rats i.v. injections of C-14 labelled $CaNa_2EDTA$ and examined certain organs autoradiographically 24 hours later. The kidneys showed accumulation in the proximal tubules but not in the glomeruli; the duodenum showed activity in the mucosa and crypts. Lesser activity was evidenced in the liver parenchyma, bile ducts, and blood vessels. The pancreas and adrenals showed no accumulation.

Plagne *et al* (1969) injected C-14 labelled HgEDTA into rats and found accumulation only in the renal cortex.

Tanno *et al* (1972) gave rabbits i.v. dosage of In*EDTA and measured the disappearance with time from the blood and organs. After 30 minutes the accumulation was (in decreasing order): kidney, blood, lung, pancreas, liver, marrow, spleen, brain. All decreased at about the same rate except the pancreas which increased up to 100 minutes before dropping.

B. Physiological Effects

Kabakow and Brothers (1958) gave a number of adult human subjects an i.v. injection over a four-hour period of 4 gm Na_2EDTA in 250 cc of 5% aqueous dextrose. On average the serum Ca was depressed 1.9 mg%. About half the time minor hypotension resulted, at most 15 mm systolic, 10 mm diastolic. Throughout the infusion period a tolerable, burning pain was felt from the point of insertion and downstream. Accompanying this in 1/3 of the subjects was a sensation of prickling around the mouth and warmth elsewhere on the face.

Vozar and Bobek (1958) gave oral doses of Na_2EDTA to guinea pigs and rats, resulting in strong decrease of γ-globulin. Zizine (1958) reported that feeding rats $CaNa_2EDTA$ as 1/2% of their diet significantly reduced the thyroid activity. Fujita and Imai (1958) gave rats 10 daily injections of 50 mg/kg of $CaNa_2EDTA$, or 11 doses of 5 mg/100 gm of Na_2EDTA. Results of the Ca treatment were: decrease of HIO_4-Schiff positive substances in the heart, regressive degeneration of the liver, degeneration of the kidney, slight congestion with many large nuclear cells in the spleen, and hemorrhage with wide cells in the lung. These results were repeated after the other treatment but to a lesser extent; in addition the vascular and lymphatic systems and capillaries showed lesions and bleeding. Sacca *et al* (1958) thought that the side effect, osmotic nephrosis, from treatment of Pb poisoning with $CaNa_2EDTA$ derived from the Na atoms.

Vozar (1959) studied the effect of Na_2EDTA on Cu in rats. Feeding 40-80 mg/100 g/d for three days produced a marked decrease of hepatic, renal and skeletal musculature Cu content. Running the experiment for 20-40 days resulted in deposition of Cu in skeletal muscle and cerebral

cortex gray matter concurrently with removal from liver, kidneys and heart.

Kelenyi and Kasza (1959) gave rats a 60 mg dose of Na_2EDTA by interscapular injection, resulting in the development of a tumor-like edema at the injection site, attributed to the binding of Ca.

Vozar and Simko (1959) examined the blood of rats dosed with 40 mg/100 g/d of Na_2EDTA for 3-7 weeks. After three weeks there was no change in the number of segmented neutrophile leucocytes or erythrocytes, but the latter's hemoglobin content had decreased. The number of lymphocytes and the total leucocyte count also decreased. The blood gradually returned to normal during the 4th-7th weeks of treatment.

Foreman (1959) reported that EDTA parenterally administered to rats caused hydropic degeneration of renal proximal tubules, reversible on cessation of dosing.

Spencer (1960) reported that i.v. dosage of humans with 4 g Na_2EDTA over a four hour period produced in the urine only about 65% of the extra Ca which the EDTA was capable of complexing, without affecting the serum Ca level. A similar test with CaEDTA produced only 81% of the expected excess in the one day test period.

Sullivan (1960) reported that a diet containing 4% MnEDTA caused a reversible, severe iron deficiency anemia in immature, but not in adult, rats.

Smith and Kerby (1960) gave rabbits a number of subcutaneous injections of Na_2- or $CaNa_2EDTA$ resulting in urinary excretion of acid mucopolysaccharides of brief and widely variable duration and extent.

Albach (1961) reported that the effect of Na_2EDTA on serum Mg level on human males was a function of age. Below 25 years no effect was

observed; above, there was an average drop of 37% in two hours after injection. Normal levels recovered within 12 hours.

Remagen *et al* (1961) gave daily injections of 150-200 mg/Kg EDTA to young rats and rabbits for 14 days. Results included significant lowering of serum Ca and serum alkaline phosphatase, considerable Ca excretion, and change of blood pH to an alkaline condition.

Oser *et al* (1963) reported that feeding rats for two years and dogs for one year on a diet containing up to 250 mg/Kg of body weight had no effects on physiological responses or mineral metabolism.

Daniel and Erwin (1965) reported that Na_4EDTA had a stronger effect than Na_2EDTA on the contraction and relaxation of rat uterus which depend on Ca and Mg ions.

Schane (1965) gave spayed rats i.p. doses of 0.6 mmole/Kg EDTA and found that within one hour there was an increase in uterine phosphorylase activity similar to that seen 48 hours after estradiol treatment. Similar treatment of cows, guinea pigs, mice, and rabbits gave intraspecies inconsistency.

Neu *et al* (1966) demonstrated that the combination of EDTA and Tris-HCl effected the release of acid-soluble nucleotide material from E. coli in 6-10 minutes. The viability of the cells was unchanged.

Hamilton-Miller (1966) demonstrated that Na_3EDTA and Na_4EDTA, but not $CaNa_2EDTA$ or MgEDTA, greatly increased the outer membrane permeability of various common bacteria at concentrations as low as 0.1 mM, without impairing the viability. Nucleic acid material leaked from the cells. The maximum effect was found to occur at pH 7.4-7.6, coincidentally, perhaps, the optimum pH for chelation of Ca by EDTA.

Watras *et al* (1966) gave rabbits i.v. doses of 20 mg/Kg of $CaNa_2EDTA$

on alternate days for 48 days. There was a significant reduction in the amount of Fe stored in the liver, spleen, and long bone marrow.

Rasmussen and Cooper (1968) and Cooper *et al* (1968) reported that ethylene production in the calamondin (citrus) tree was stimulated by CuEDTA, but not by FeEDTA.

Schwiegel (1969) found that the permeability of the main capillaries of rabbits and rats to the dye Evans blue was increased not at all by $CaNa_2EDTA$, somewhat by $MgNa_2EDTA$, more so by Na_2EDTA.

Dubina *et al* (1969) reported that seven consecutive daily i.p. injections of 70 mg/Kg of EDTA to adult rats temporarily decreased the liver mitochondria activity but did not affect the activity of the respiratory enzymes dependent upon Cu or Fe. Different results had been found in an in vitro study.

Fiedler and Hartmann starved guinea pigs for 16 hours, then gave them s.c. injections of 0.3 mmole/Kg EDTA, 0.6 mmole/Kg MgEDTA, 0.15 mmole/Kg ZnEDTA, 0.6 mmole/Kg ZnEDTA, 0.03 mmole/Kg Zn_2EDTA. They then measured serum glucose at 1/2, 1, 1 1/2, 2, and 3 hour intervals. EDTA itself elevated the glucose level by 29% at 1/2 hour, and prevented return to normalcy by 2 hours. Co-injection of an equivalent amount of alloxan (non-hyperglycemic and non-diabetogenic in guinea pigs) caused the glucose to rise 50%, and remain up 30% at 3 hours. The effect of the Mg and Zn EDTA's was less marked. It was concluded that the EDTA acted by epinephrin secretion stimulation, and not by Zn complexation.

Lie and Brotonegoro (1969) found that FeEDTA, and K_2EDTA to a lesser extent, interfered with nodule formation on the roots of pea plants.

Wynn (1970) fed rats varying amounts of Na_2EDTA for three months,

with the effects on food consumption and weight gain in Tables VIII and IX, respectively.

Table VIII. Effect of EDTA on Average Weekly Food Consumption in Adult Male Albino Rats

	Diet consumed (g)			
		Na_2H_2EDTA		
End of week	Control	1.0%	5.0%	10.0%
1	141	141	111	116
2	164	171	137	98
3	169	158	129	85
4	174	162	108	76
5	168	193	115	61
6	152	147	122	86
7	175	162	134	69
8	165	178	145	78
9	166	177	148	85
10	163	174	157	80
11	165	182	158	71
12	151	144	135	84
13	145	137	144	53

Table IX. Effect of EDTA on Average Weekly Body Weights of Adult Male Albino Rats

	Body weight (g)			
		Na_2H_2EDTA		
End of week	Control	1.0%	5.0%	10.0%
0	116	121	120	123
1	171	171	130	116
2	223	218	156	119
3	273	255	177	119
4	313	286	185	124
5	334	310	188	115
6	351	320	203	115
7	373	335	209	119
8	390	361	235	122
9	406	380	248	129
10	422	403	267	145
11	431	399	261	146
12	437	409	276	136
13	442	418	305	127

In both tables differences in only the 5 and 10% columns have statistical significance. Animals on the two higher concentrations developed diarrhea by the third day on the diet and were subject to it throughout the experiment, consuming twice as much water as the controls. Some of the animals

were subject to priapism (all of the 10% and one-fifth of the 5%) onsetting in the initial four weeks. There were no hematological differences at the end of the test. Gross and histopathological examination of the internal organs showed no abnormalities, other than pale livers in the 10% group.

Vohra and Bond (1970) fed fowl diets ranging 0.5-4.0% in $Na_2EDTA \cdot 2H_2O$. The high levels depressed weight gain, hematocrit levels, Fe levels in the blood, liver, and kidney, and Zn level in bones; renal Zn was increased.

Dvorak (1970) found that increased presence of K and Mg ions in the urine of rats dosed i.p. with Ca- or $ZnNa_2EDTA$ was solely a function of the Na ions in the dose.

Fritz *et al* (1971) fed chicks diets containing various amounts of EDTA, Na_2EDTA, and $CaNa_2EDTA$ to study the utilization of dietarily marginal amounts of Ca, Fe, and Mn. Amounts up to 1600 ppm had no effect on the Ca. Incidence and severity of perosis from 1600 ppm of Na_2- or $CaNa_2EDTA$ or 800 ppm of EDTA were equivalent. The same amount of EDTA depressed growth and hemoglobin. Depression of hemoglobin and hematocrit also resulted from 800 ppm of the two EDTA salts.

IX. ENVIRONMENTAL EFFECTS

A. Persistence and/or degradation

Hill-Cottingham and Lloyd-Jones (1957) reported that FeEDTA was rapidly adsorbed on calcareous clay soil. Most of the EDTA remained water extractable but the Fe precipitated after being exchanged for Ca. Hemwall (1958) agreed that clay minerals do not retain EDTA but will precipitate Fe from FeEDTA. Moawad (1970) studied extractability of FeNaEDTA from four different soils and found that the more acid the

soil the longer the Fe remained water soluble.

Cheronis and Schatz (1958) commented that EDTA was known to be very resistant to degradation by soil microfauna and -flora. Bunch and Ettinger (1967) tested the ability of sewage to degrade EDTA. Over a three week period there was little or no apparent degradation of concentrations ranging from 5-20 mg/1. Rudling (1972) analyzed for EDTA in samples from several sewage works and found that the content was the same in the effluent as in the input - indicative of no degradation.

B. Environmental transport

Knuttson and Forsberg (1966) applied CrEDTA to columns of 20 minerals, 5 rock types, and 6 soil types. There was no absorption on quartz, feldspar, calcite, dolomite, and some micas. Clays, chlorite, Fe(II)-Mg silicates and other Fe minerals retarded the EDTA somewhat. Nishita and Essington (1967) studied the movement of EDTA through five soils of widely different chemical and physical properties. Moawad (1970, pp 91-100) did a similar study on FeNaEDTA.

C. Bioaccumulation

No indications of bioaccumulation or concern about it were found.

X. TOXICITY

A. Human

Clarke *et al* (1955) reported on the side effects of human EDTA therapy. Almost two dozen patients received 10-100 doses of Na_2- or K_2EDTA, 5 gm, delivered i.v. in 500 cc of 5% glucose or normal saline over a 1 1/2-3 hour period. The use of the K salt was terminated because of intolerable burning sensations at the puncture site and downstream. The Na salt also caused burning but at a tolerable level. Other effects included nausea, diarrhea, dermatitis.

Vinerga (1956) reported that therapy using "non-consecutive" daily doses of 1.5 g $CaNa_2EDTA$ had the potential of causing sensitization to the EDTA, and, consequently, was not recommended.

Kabakow and Brothers (1958) commented that they were able to find only one literature report of EDTA-caused human fatality. In this instance two patients received daily doses of 28-40 g, apparently dying from kidney failure.

Meltzer *et al* (1961) reported the results of 2,000 Na_2EDTA treatments involving 81 patients. In Table X is their patient-treatment distribution. In Table XI is the actual number of occurrences of the indicated side effects (incidences of these side effects had been reported in Seven and Johnson (1960)). A 3 g dose in 500 ml of normal saline or glucose solution was administered over 2 1/2-3 hours. Doses were given every other day until 20 had been given; then a 6-8 week period of no treatment was initiated before further treatment, if any.

Foreman (1963) reviewed literature reports on side effects from EDTA therapy.

TABLE X—DISTRIBUTION OF PATIENTS ACCORDING TO TOTAL NUMBER OF INFUSIONS

Number of Patients	*Total Number of Infusions*
19	1–10
30	11–20
19	21–40
9	41–60
1	61–80
3	81–120

TABLE XI–SIDE EFFECTS OF EDTA ADMINISTERED WITH 2000 INFUSIONS

Side Effect	*Frequency of Occurrence*
A. RENAL DAMAGE (increase in BUN, decrease in PSP excretion, cylindruria, hematuria, or persistent albuminuria greater than 1+	0
B. BURNING AT INJECTION SITE or along course of vein:	
Initially only,	30
Throughout infusion,	68
C. THROMBOPHLEBITIS	1
D. HYPOTENSION: Mild: a drop of systolic pressure of 20 mm. without symptoms	8
Moderate: a drop of systolic pressure of 30 mm. with or without symptoms	23
Severe: a drop of 30 mm. or more with distinct hypotensive symptoms	2
E. HYPOCALCEMIA: Mild: numbness, tinkling at circumoral area or leg cramps or muscle spasm	20
Severe: signs of tetany (Chvostek's sign, and others) or fall in serum calcium to 7 mg./100 ml.	0
F. SYSTEMIC REACTIONS (febrile reaction, malaise, fatigue, headache, anorexia)	0
G. HISTAMINE-LIKE REACTION (sneezing, lacrimation, nasal congestion)	0
H. ANEMIA or other hematopoietic changes related to treatment	0
I. GLYCOSURIA OR HYPERGLYCEMIA	0
J. DERMATITIS (presumably due to pyridoxine deficiency)	0
K. NAUSEA + VOMITING: Mild	15
Moderate	1
Severe	2
ABDOMINAL CRAMPS OR PAIN	2

Raymond and Gross (1969) found that EDTA at the level used as a preservative in ophthalmic solutions was responsible for some cases of acute allergic conjunctivitis and periorbital dermatitis. They also found evidence for cases of delayed hypersensitivity.

B. Birds and Mammals

Coune and Driggers (1954) gave male chickens doses of Na_4EDTA ranging from 50-200 mg/Kg i.v., i.p., i.m., or s.c. Only the i.v. method, at 200 mg/Kg, proved fatal. Slow, two minutes, or rapid, 30 seconds, injection made no difference. Death was attributed to lowering of serum Ca.

Toyota and Shibata (1956) reported LD-50 values for EDTA salt in mice as 20.5 mg/Kg oral and 2.6 mg/Kg i.p.

Shibata (1956) reported LD-50 values for EDTA salt in the rabbit as 47 mg/Kg i.v. and 2.3 g/Kg oral; however, extending the i.v. delivery to 10 minutes resulted in no fatalities. In tests of chronic toxicity it was found that 1 g/Kg orally for one week was fatal, but 1/2 g/Kg for one month was non-fatal. Also non-fatal was a daily i.v. dose of 20 mg/Kg as a 5% solution.

Shibata (1957) reported the i.p. LD-50 values in mice for CaEDTA as 7,600 mg/Kg, and for PbEDTA as 7,500 mg/Kg.

Köcher *et al* (1958) found that the LD-50 in mice for $CdNa_2EDTA$ was 63 mg/Kg i.p.

Köcher *et al* (1959) found that the LD-50 in mice for $NiNa_2EDTA$ was 1,244 mg/Kg.

Paulet *et al* (1959) found that the LD-50 in mice for Co_2EDTA was 50 mg/Kg i.v.

Eybl *et al* (1959) found that the LD-50 in mice for $CoNa_2EDTA$ was 1,948 mg/Kg i.p.

Sykora *et al* (1960) found that the LD-50 in rats for $MnNa_2EDTA$ was 1,930 mg/Kg as a 10% solution i.p. with a ten-day observation period.

Toyoda (1960) found the following i.v. LD-50's in mice: Na_4EDTA 60 mg/Kg, Na_2EDTA 460 mg/Kg, $CaNa_2EDTA$ 3,250 mg/Kg.

Nofre *et al* (1962) found the LD-50 in mice for $FeNa_2EDTA$ was 281 mg/Kg i.p. in 30 days.

Kostial *et al* (1962) found the LD-50 in adult female rats for EDTA was 397 mg/Kg i.p. in one day, or 350-450 mg/Kg with a 95% confidence limit. Symptoms were severe within 10 minutes and consisted of distress signs and hypocalcemic convulsions, with death normally occurring in two hours.

Nofre *et al* (1963) reported LD-50's for rapid i.p. dosage in adult male mice of $Na_2EDTA \cdot 2H_2O$ and various MNaEDTA's. The 30-day values, in order of increasing toxicity, were (mg/Kg): $CaNa_2$ 5,351; $MnNa_2$ 2,335; CrNa 2,034; $PbNa_2$ 1,678; $CoNa_2$ 1,376; $NiNa_2$ 589; $ZnNa_2$ 519; $Na_2 \cdot 2H_2O$ 298; $FeNa_2$ 281; AlNa 183; FeNa 139; $CdNa_2$ 31; $CuNa_2$ 13; $HgNa_2$ 7. Comparing the LD-50's (from a therapeutic viewpoint) of the metal chelates with the metals alone it was found that, on a weight of metal basis, the Al, Cu, and Hg chelates were more toxic, the Fe(II) and Fe(III) chelates were equally toxic, and the others less toxic.

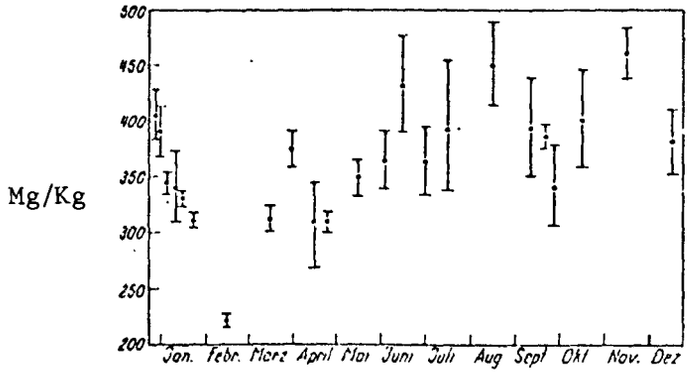
Oser *et al* (1963) reported oral LD-50's for $CaNa_2EDTA$ in fasted animals as 7 g/Kg in rabbits, 10 g/Kg in rats, and 12 g/Kg in dogs.

Osanai *et al* (1964) allowed mice to drink water containing varying amounts of $CaNa_2EDTA$. At 4% the mice died in one week suffering from diarrhea; at 2% the mice died in seven weeks suffering from anemia; at 1% the mice survived at least 21 weeks suffering only slight anemia. There did not appear to be any nephrotoxicity.

Bekemeier (1965) found that the s.c. LD-50 for Na_2EDTA in mice was far from being even relatively constant over a one year period. The

results are reproduced in Graph III.

Graph III. Subcutaneous LD-50 of Na_2EDTA as a Function of the Time of Year



Cier and Abecassis (1966) studied the genesis in adult male mice of diabetes mellitus by Na_2EDTA and ZnEDTA. A 40 mg/Kg i.p. dose of Na_2EDTA caused symptoms to appear after five days; these were gone after 31 days. The highest dose tested, 300 mg/Kg, did not elicit symptoms until 17 days, but they persisted after 31 days in 45% of the animals. Giving 30 mg/Kg doses on three consecutive days proved to have additive effects. A 40 mg/Kg dose of ZnEDTA gave the same results as that amount of Na_2EDTA in a one week period, but then the glycemia rapidly regressed.

Fiedler (1969) reported that $CuNa_2EDTA$ had a higher toxicity than Cu(II), or $CaNa_2EDTA$, in guinea pigs, rabbits, and rats. Within eight hours of an i.v. injection of 12.7 mg/Kg in rabbits of $CuNa_2EDTA$ there were 30-250 fold increases in the serum plasma activities of these enzymes: alanine and aspartate aminotransferase, glutamate, sorbitol and lactate dehydrogenase, and fructose diphosphate aldolase. Serum levels of K increased, Ca decreased, and Na didn't change. In all three species blood sugar fell to a very low level (after an initial rise in guinea pigs).

Wynn *et al* (1970) found that rats fed diets consisting of 5 and 10% Na_2EDTA suffered 20 and 60% mortalities, respectively, with the first death in the higher group occurring in the third week (the study ran for 13 weeks).

Lenza (1971) reported an i.v. dose of 25 mg/Kg of SbEDTA was lethal to dogs, causing extreme diastole of the heart.

Ishmel *et al* (1971) gave sheep single s.c. doses of CaCuEDTA and found that 18 mg/Kg was the LD-50 in three days. Post mortems showed excessive fluid in serous cavities, edema of the lungs, mottled livers, congested kidneys, and subendocardial hemorrhage. Histological examination showed hepatic centrilobular congestion, hemorrhage and necrosis.

Swenerton and Hurley (1971) fed pregnant rats diets containing 2 or 3% Na_2EDTA through all or part of the gestation period. At the lower level for the whole period litter size was normal, but the newborn were smaller than normal and 7% were malformed. At the higher level for the whole period all fetuses had been resorbed. At the higher level for the 6-21 day portion litter size was less than half the normal, all newborn were very small and all were grossly malformed. Results similar to the 6-21 day period were seen when the higher dose was given during the 6-14 day period.

C. Lower Animals

van Asperen and van Esch (1956) injected cockroaches with 30 µl of 67 mM EDTA solution. Within one day there was 30% mortality (the remaining insects recovering). In the initial 15 minutes appeared symptoms of paralysis and intoxication, all traces of free Ca in the haemolymph having disappeared.

Khristolyubova (1961) incubated Drosophila eggs in a nutrient medium

containing EDTA. Mortality in two-three days was 50%. All hatched adults were stunted. An above normal number of nucleoli was present in the salivary gland chromosomes.

Terriere and Rajadhyakshia (1964) found that spider mites produced fewer offspring when feeding on leaves treated with various EDTA metal complexes.

Ulitzur and Shilo (1966) reported an LD-100 for minnows immersed in a 0.3 mM EDTA solution (about 80 ppm).

Sell and Schmidt (1968) reported that concentrations in the diet of cabbage loopers of as low as 0.05% EDTA delayed development and caused developmental aberrations on occasion; at 0.5% pupation was completely suppressed.

Brahmarchary *et al* (1968) found that 5mM EDTA stopped cleavage of eggs of Lymnaea (fresh-water snail) after one hour exposure.

Noble (1970) reported that cell aggregation and change from bladder to filiform amebocyte in the sea cucumber was prevented by the presence in the water of EDTA at pH 6, but at pH 7.8 only the cell aggreation didn't occur.

D. Plants

Sussman (1954) found that ascospores of Neurospora tetrasperma were inhibited at the germinating stage when in the presence of 3.5 mM EDTA, but when dormant or only newly activated were insensitive to much higher concentrations. It was demonstrated that the EDTA was not penetrating into the spore.

Eversole and Tatum (1956) found that different strains of an alga either incurred increased mutation from contact with EDTA prior to mating, or were unaffected.

Shannon and Mohl (1956) found that EDTA at 800 ppm in a nutrient solution was toxic to bush beans after three weeks.

Delaunay (1958) found that EDTA caused chromosome crossovers in spores, and seemed to stabilize chromosomes broken by x-rays.

Marlatt (1959) studied the effect on lettuce of various metal-EDTA complexes applied in different ways. Yields were reduced by soil application of 224 Kg/hectare of Ca- or ZnEDTA. Spraying the plants with 2.3 g/l of FeEDTA burned them, and with 4.6 g/l of ZnEDTA killed some.

Michaelis and Rieger (1963) reported that immersion of the roots of Vicia faba in 1 mM EDTA for 20 hours produced 3.5 times as many chromatid aberrations as normal.

Baranauskaite and Rancelis (1966) soaked horse beans for 15 hours in 0.02 or 0.2% EDTA solutions before soil planting. At the higher concentration germinating ability and rate of germination were reduced.

Tsarapkin (1966) confirmed Delaunay's finding (above) that EDTA stabilized fragmented chromosomes, which can lead to increased mutation.

Rancelis and Luksa (1967) found that chlorophyll mutations were present in the 2nd and 3rd generations of horse beans which had been treated with 0.02-0.2% EDTA.

In a study of the effect of EDTA on nuclear division in Triticum vulgare, Retezeanu (1968) found that a concentration of 9 mM was sufficient to fragment the chromosomes during anaphase and telophase.

Matsuda (1968) found that EDTA at over 5 ppm retarded root growth

and decreased yield of rice plants.

Dumitrescu and Retezeanu (1970) found that roots of Lupinus albus treated with 1 mM Na_2EDTA suffered from nuclear fragmentation.

Joshi and Patil (1971) treated Bryophyllum pinnatum with 0.05-20.0 mM Na_2EDTA, and then allowed C-14 labelled CO_2 to be incorporated in sunlight for one hour. Analysis of the plant for various compounds and amount of C-14 therein gave the results in Table XII. Plants in 20 mM solution died in a few days.

TABLE XII. EFFECT OF EDTA ON THE DISTRIBUTION OF RADIOACTIVITY IN DIFFERENT FRACTIONS FOLLOWING $^{14}CO_2$ LIGHT FIXATION IN LEAVES OF *B. pinnatum*

(Values of incorporation of radioactivity in individual compounds are expressed as percentage of total activity counted on chromatograms while the rate of fixation is expressed as counts/min/mg fresh tissue)

Compound	Control	EDTA conc.			
		50 μM	0·005M	0·01M	0·02M
		SUGARS			
Glucose	9·9	2·31	2·69	1·18	1·92
Fructose	4·57	1·64	1·73	—	9·2
Sucrose	34·19	43·54	1·92	0·36	1·02
Total	48·66	47·49	6·34	1·54	12·12
		SUGAR PHOSPHATES			
Sugar diphosphate	2·76	0·48	0·32	0·11	0·51
Sugar monophosphate	—	0·6	0·64	—	0·76
Phosphoenol pyruvate + phosphoglyceric acid	—	2·67	1·6	0·05	1·27
Total	2·76	3·75	2·56	0·16	2·54
		AMINO ACIDS			
Aspartate	13·52	2·55	1·47	0·11	2·81
Glutamate	7·71	0·91	2·56	0·15	2·3
Glycine-serine	—	1;51	2·94	0·55	6·39
Alanine	5·01	6·13	5·12	0·67	31·84
Threonine	—	—	4·98	—	—
Leucines	—	2·88	1·53	0·45	17·38
Total	26·27	14·28	18·60	1·93	60·72
		ORGANIC ACIDS			
Citrate	15·62	16·73	62·33	93·13	9·59
Malate	1·52	1·67	0·7	1·99	7·92
Succinate	1·61	15·58	1·98	0·33	4·6
Fumarate	3·23	0·66	2·24	0·11	2·43
Total	21·98	34·64	67·35	95·56	24·54
Rate of fixation	209	220	189	405	27

E. Micro-organisms

Ujiie (1959) found that at pH 6-8 EDTA was not toxic to E. coli over 48 hours of contact, but it did inhibit propagation.

Nezval (1964) found that EDTA was synergistic with the bacteriocide Septonex against Pseudomonas aeruginosa. On a concentration basis a solution containing one part of Septonex to two of EDTA was ten-fold as effective after five minutes exposure as a solution containing only the same amount of Septonex.

Patel and Shah (1965) tested the antifungal and bacterial activity of 2% Na_2EDTA against that of penicillin, streptomycin, and various chemicals of simpler structure. Against nine gram-positive bacteria, the EDTA was about 60% as effective as 5 µg/ml of the K salt of penicillin; against three others the EDTA was at least as effective. Against six gram-negative bacteria, the EDTA was about 80% as effective as 20 µg/ml of streptomycin sulfate; against four others the EDTA was 100-130% as effective. Against 0.25% methyl paraben the EDTA was more effective in eleven of twelve fungi tested; against 0.5% benzoic or salicylic acid the EDTA was slightly more effective in eight of twelve (not the same eight).

Goldschmidt *et al* (1967) found that the male strains of E. coli were far more sensitive than the female to a mixture of EDTA and Tris.

Neu (1969) could not completely confirm Goldschmidt's results, finding closer toxicity between male, female, and Hfr strains.

Nezval and Ritzerfeld (1970) found that EDTA was synergistic with chloramphenicol or neomycin, but not with carbenicillin or gentamycin, against Pseudomonas aeruginosa.

Russell (1971) reviewed the antibacterial activity of EDTA.

XI. CURRENT REGULATIONS

No information other than the FDA limitations given in Section III was found.

XII. STANDARDS

No information was found.

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FORMALDEHYDE RESINS

SUMMARY AND CONCLUSIONS

The finished products made from the thermosetting resins resulting from the reactions of formaldehyde with urea, melamine, or phenol are infusible, insoluble, hard and mar-resistant, flame-resistant, and are chemically inert under use-related conditions.

The finished products made from the thermoplastic resins resulting from the reactions of formaldehyde or trioxane with ethylene oxide have high strength and rigidity, good electrical properties, abrasion resistance, good flame resistance, and are chemically inert under use-related conditions.

The expected trend in the production of the formaldehyde resins is upward. Some set-backs in the production and sales of the formaldehyde resins have been experienced because of the energy crisis and shortages of starting materials, and because of a decrease in demand from some manufacturing areas. These set-backs are considered by the plastics industry to be cyclical, however, and the overall trend in the manufacture and sales of these resins is expected to be upward. In fact, an annual growth rate of 6.1% per year has been predicted by the plastics industry, from the present time to the year 2000.

The versatile formaldehyde resins have usually wide application ranges. The amino resins are used in closures and wiring devices, large and small appliance housings, dinnerware, buttons, ash trays, and utensil handles. They are used as adhesives in plywood and in laminating. In textile treating, they are used for greaseproofing, water

repellancy, and flame retardance. In paper treating, they are used for improving wet strength, rub resistance, and dry tensile values.

The acetal homopolymer is used to replace metal parts in the plumbing industry. It is used in truck-trailer connectors and in such a variety of items as furniture casters, hardward items, bodies of lighters, replaceable cartridges in shavers, toy components, telephone pushbuttons, and stereo-tape and cassette components.

The acetal copolymer is used in automotive gears and fuel-emission systems. It is also used to give satiny surfaces and hardness to pen barrels and other items where an attractive appearance is desired. Its dimension stability qualifies it for use in aersol containers under continuous pressure.

The phenolics are used as adhesives in the wood particle board used in building panels and furniture, and as a water-resistant glue for exterior grade plywood. They are used extensively as automotive components in transmissions, distributor caps, coil towers, rotors, fuse blocks, for brake linings, clutch parts, and transmission bands.

Because of the tremendous variety of uses that have been found for the formaldehyde resins, it is almost impossible to avoid daily contact with products which have been manufactured from them. The fact that there are no reports in the collected literature concerning toxic effects from contact with any of these products would certainly verify that the formaldehyde resins are physiologically inert in the finished state.

They are not biodegradable, and they persist in their solid form under normal atmospheric conditions.

During resin manufacturing, however, certain starting reagents, fillers, and resin dusts present a real hazard to workers who are without adequate protection. While urea and melamine have no history of toxicity, nor have they been known to be a source of occupational problems, formaldhyde, phenol, and asbestos (a filler) are toxic. Formaldehyde is a sensitizing agent and a mucous membrane irritant. Phenol is highly corrosive to the skin and produces severe burns. Asbestos, if introduced into the respiratory tract, causes emphysema and neoplasms of the lung. Granulomas were found in the lungs of rats which had been subjected to the inhalation of the dust of acetal resin; granulomas were also found in the subcutaneous tissue and in the peritoneal area of rats after acetal powders had been injected at these sites. All of the reports in the literature which dealt with toxic symptoms in workers were written outside of the United States.

In fact, in all of the articles written in the United States concerning the safety of workers in the plastics industry, the stress was placed on equipment safety. This situation is about to reverse, however, because of the recent indictment of two chemicals which are used in industry, and with which carcinoma has been associated. Formaldehyde is directly related to one of these chemicals - chloromethyl methyl ether, which has been shown recently to cause malignant lung neoplasms. Formaldehyde in contact with hydrochloric acid will yield chloromethyl methyl ether. Conditions for this reaction were not stated in the cursory news medial report (when formaldehyde and hydrochloric acid are used for chloromethylation in the laboratory, a zinc chloride catalyst is used).

The question might be raised here as to whether workers in the United States have been well protected by clean factory operations and by compliance with the emission limits which have been set for basic raw materials, resins, and compounds, or whether the toxic hazards have been known, but experience and clinical investigation are just now producing sufficient documentation to put the hazards into proper perspective.

FORMALDEHYDE RESINS

I. PHYSICAL PROPERTIES

A thermosetting resin is a crosslinked, polymeric material which has been rendered substantially infusible and insoluble by curing it with heat or with chemical catalysts. Urea-formaldehyde resins, melamine-formaldehyde resins, and phenol-formaldehyde resins are included in this group.

A thermoplastic resin is a material with a linear macromolecular structure which will repeatedly soften when heated and harden when cooled. The acetal homopolymers and the acetal copolymers are included in this group.

A. AMINO RESINS

Amino resins are thermosetting condensation polymers formed in the reaction between formaldehyde and organic compounds which contain more than one $-NH_2$ group per molecule. Urea-formaldehyde resins and melamine-formaldehyde resins are the most commercially significant compounds of this group.

The physical form of these reaction products may be either fluffy powders or dense granules. Specific fillers may be added to meet specific requirements. The addition of alpha cellulose, for instance, enhances strength, moldability, and dimensional stability.

Alpha cellulose-filled compounds are translucent. The addition of this filler to urea and melamine molding compounds yields moldings with an attractive gloss. They are quite hard and mar-resistant. Molded items do not collect dust by build-up of a static electrical

charge and so do not require the addition of an antistatic agent. Both urea and melamine compounds are resistant to oils, solvents, and greases. They are intrinsically flame-resistant and have good electrical arc resistance. They exhibity high-heat distortion temperatures, good strength properties, and are odorless and tasteless.

Substitution of alpha cellulose by other fillers enhances specific physical or electrical properties; this is usually at the expense of appearance, handling properties, or molding properties, however. Wood flour, cotton fabric, asbestos, and glass fibers are alternative fillers for these compounds.

B. ACETAL RESINS

Acetal resins are thermoplastic resins containing the following repeating unit: $-CH_2-O-$.

These resins are produced from formaldehyde or trioxane (a cyclic trimer of formaldehyde), either as homopolymers of formaldehyde or as copolymers of trioxane with other organic compounds (e.g., ethylene oxide).

High strength and rigidity, dimensional stability, and resilience are some properties of these compounds. Acetal homopolymers are available in a number of compositions to fit a variety of end-use requirements. These compositions differ primarily in melt viscosity. Mechanical properties of the various grades are similar except for tensile elongation and impact strength. Acetal homopolymer has a tensile strength at room temperature of 10,000 p.s.i. with no true yield point and a flexural modulus of 410,000 p.s.i. Acetal homopolymer has out-

standing creep resistance (creep is a time-related change in dimension of a material under load). Its fatigue endurance at room temperature is 5000 p.s.i. It is resistant to organic solvents, although contact with strong acids or strong bases is not recommended. It has good abrasion and frictional resistance with hardness and resistance to scratching. It maintains good electrical properties under high temperature and humidity exposure, after immersion in water, and on aging.

Acetal copolymer compares with die-cast metal in its resistance to creep under load at elevated temperatures. It has excellent electrical properties, low moisture sensitivity and high solvent and alkali resistance. It is attacked by oxidizing agents and acids, however. Samples in boiling water retain nearly original tensile strength for six months, but for maximum long-term continuous use, the recommended temperature in water is 180° F.

C. PHENOLIC RESINS

Phenol and aqueous formaldehyde are reacted in the presence of alkaline or acid catalysts to produce both liquid and rigid resins (the phenol-formaldehyde resins). These have excellent dimensional stability, heat resistance which is superior to most other thermosetting materials, high heat-deflection temperature, outstanding creep resistance, and good flame resistance. Almost all phenolics for years have been rated nonburning according to ASTM D635. More recently, certain phenolics received formal self-extinguishing Group I ratings according to Underwriters Laboratory Bulletin 94. A further discussion of flammability ratings is given in Section XII, B,

General purpose materials with wood flour as the main filler are used im most applications where the basic property profile of phenolics

is adequate. Where higher heat-resistance is required, the wood flour is replaced by mineral-filled compounds (such as asbestos).

D. VINAL FIBERS

Fibers based on the reaction product of polyvinyl alcohol and formaldehyde are known as vinal, vinylon, or PVA fibers. Fabrics made from these fibers have a cotton-like feel. They are strong, abrasion resistant and moisture-absorbent, and are quick-drying and inexpensive. However, they cannot be dyed in bright colors, they are susceptible to shrinkage, and they cannot be heat-set. Vinal fabrics soil easily, have poor elasticity, and wrinkle readily. It is for these reasons that vinal has not been successful as an apparel fiber. However, it has industrial applications because of its high strength, durability, and resistance to weathering, heat, and abrasion. The fibers have excellent adhesion to plastics, usually without the need for coupling agents. They are light-weight with a density of 1.25. They do not break under high pressure and shear of injection molding (Modern Plastics Encyclopedia, 1973-74; Chemical Economics Handbook).

II. PRODUCTION

A. AMINO RESINS

Some of the leading manufacturers of amino-formaldehyde resins in the United States are American Cyanamid Co. (Cymel, Urac, Melurac); Allied Chemical Corp. (Plaskon); Rohm and Haas Co. (Uformite, Phonite), and Borden Chemical Co. (Casco).

The following production figures have been calculated from data reported by several sources.

ESTIMATED PRODUCTION FIGURES
(Metric Tons)

	UREA RESINS	MELAMINE RESINS
1964	190,000	69,000
1965	225,000	78,000
1966	250,000	95,000
1967	234,000	97,000
1968	315,000	94,000
1969	343,000	95,500
1970	321,000	117,700

In 1971, urea and melamine sales increased by about 15% over 1970. Their combined sales again increased by 15% in 1972. The sales figures for 1973 show an increase of 13% over 1972, which is a 2% drop from the previous year's growth rate. Further production figures for urea-formaldehyde resins and melamine-formaldehyde resins, based on consumption and applications, are included in Section III, USES.

B. ACETAL RESINS

Acetal resins are produced in the United States by two companies: Celanese Corporation, Celanese Plastics Company Division, Bishop, Texas; and E. I. duPont de Nemours and Company, Inc., Plastics Department, Parkersburg, West Virginia, according to Chemical Economics Handbook.

The trade name of the Celanese product is Celcon, which is an acetal copolymer. Plant capacity was estimated at about 70 million pounds per year in mid-1971.

The trade name of the duPont product is Delrin, which is an acetal homopolymer produced in several grades. Plant capacity was estimated at about 55 million pounds per year in mid-1971.

Since there are only two producers, separate data on the U.S. production of acetal resins have not been published by the U.S. Tariff Commission. The following estimated production figures are based on consumption estimates made by trade sources.

ACETAL RESIN PRODUCTION (ESTIMATED)
(Metric Tons)

1965	20,000
1966	26,000
1967	28,000
1968	33,500
1969	38,900
1970	39,000

In 1971, acetal resin sales increased 10% over 1970 sales. The 1972 sales increased 11.5% over 1971, and the 1973 sales attained a record advance of 18.5% over 1972.

Additional statistics for the acetal resins, based on consumption and applications, are given in Section III, USES.

C. PHENOLIC RESINS

Among the manufacturers of phenol-formaldehyde resins in the United States are Ashland Chemical Co. (Arofene, Arochem, Arotap), Borden Chemical Co., Clark Oil and Refining Corp., Firestone Tire and Rubber Co., Formica Corp., General Electric Co., Hooker Chemical Co., Rohm and Haas Co., Westinghouse Electric Corp., Union Carbide Corp. (Bakelite), and Monsanto Co. (PF-535; Resinox 517).

PHENOL-FORMALDEHYDE RESINS PRODUCTION (ESTIMATED)
(Metric Tons)

1962	263,400
1963	284,000
1964	322,200
1965	356,600
1966	405,900
1967	384,000
1968	432,200
1969	464,800
1970	462,200
1974	212 (preliminary figures, May)

In 1971, the sales of phenol-formaldehyde resins remained on a level with 1970. In 1972, new molding techniques led to an increase in the sales of phenolics of 30% over 1971; the greatest growth was in the field of appliances. Phenolics showed only a 0.4% gain in 1973 over 1972, attributable to a major drop in the plywood market.

Additional data on the production of phenolics is given in Section III, USES.

D. VINAL FIBERS

Vinal fibers are not produced in the United States. Approximately 103 metric tons were consumed in 1970, all of which were imported.

III. USES

A. AMINO RESINS

The properties of the alpha cellulose-filled urea molding materials qualify them for use in closures, wiring devices, electric blanket controls, toilet seats, stove and refrigeration hardware, knobs, buttons, and electric appliance housings. The closure applications (both straight-wall and reverse-taper types) and wiring devices (switchplates, toggles, receptacles) predominate. Allied Chemical Company's Plaskon is a typical urea resin recommended for these applications.

Alpha cellulose-filled molding compounds are used to form dinnerware, buttons, ash trays, utensil handles, electric shavers and housings. American Cyanamid Company's Cymel 1077 (a melamine-formaldehyde resin) is a representative of this group of resins.

Adhesives are manufactured from both urea and melamine resins, but the bulk of these are urea-formaldehyde resins. The melamine adhesives are superior to urea adhesives in water resistance and weathering, giving boil-resistant bonds.

As laminates, the melamine resins offer superior hardness and wear resistance; in industrial laminates their added advantages are flame, arc, and heat resistance. Some of the applications are in the manufacture of tabletops, countertops, and wall paneling.

The amino resins are used in textile-treating in creaseproofing, shrinkage control, stiffening, water repellency, and flame retardance.

In paper treating, the amino resins improve wet strength, burst strength, rub resistance, and dry tensile values.

Alkylated methylolureas and methylolmelamines are extensively used with alkyl resins in baking enamels for greater hardness, mar and chemical resistance, and durability (Modern Plastics Encyclopedia, 1973-74).

Following are consumption statistics for urea and melamine resins in metric tons:

	1971	1972	1973
Urea Resins			
Closures	6,900	6,800	7,900
Electrical devices	11,400	10,900	12,600
Melamine Resins			
Buttons	900	800	900
Dinnerware	19,100	18,200	19,100
Sanitaryware	--	500	600
Collective statistics			
Bonding and adhesive resins for:			
Fibrous and granulated wood	199,000	232,000	262,000
Laminating	22,000	24,000	24,000
Plywood	31,000	40,000	40,000
Paper treating and coating	14,000	16,000	22,000
Protective coatings	19,000	28,000	33,000
Textile coating and treating	23,000	23,000	26,000

B. ACETAL RESINS

The properties of the acetal homopolymer makes it suitable for use in the plumbing industry in shower heads, valves, and fittings, replacing brass and zinc parts. It is used in truck-trailer connectors and furniture casters. Handles and other hardware items are formed from the homopolymer, as are the bodies of lighters, replaceable cartridges in shavers, toy components, telephone pushbuttons, lawn sprinklers,

stero-tape cartridge and cassette components.

The acetal copolymer is used in automotive components such as gears and fuel-emission systems. Since it is noncorrosive in long-term hot-water exposure and can be used with metals, it finds application in the plumbing industry. Its creep resistance qualifies it for use in aerosol containers under continuous pressure. It is used to form pen barrels and other components where satiny surfaces, hardness, and stain resistance give added value and better appearance (Modern Plastics Encyclopedia, 1973-74).

Following are consumption statistics for the acetal resins in metric tons:

	1971	1972	1973
Appliances	4,820	5,600	5,900
Consumer products	3,090	3,500	3,800
Electrical/electronics	1,910	2,100	2,500
Machinery parts	2,550	2,800	3,900
Plumbing and hardware	2,550	2,800	4,000
Sheet, rod, tube	1,320	1,500	1,800
Transportation	6,050	5,700	6,500

The phenol-formaldehyde resins are used in power-brake and automatic transmission components, distributor caps, coil towers, rotors, fuse blocks, and connectors. They are used to bond friction materials for automotive brake linings, clutch parts, and transmission bands. They serve as binders for wood particle board in building panels and furniture, as water-resistant glue for exterior grade plywood, and as the bonding agent in acoustical and thermal insulation pads.

Following are consumption statistics for the phenol-formaldehyde resins in metric tons:

	1971	1972	1973
Bonding and adhesive resins for:			
Coated and bonded abrasives	8,000	9,100	11,200
Fibrous and granulated wood	30,000	40,000	42,000
Friction materials	14,000	13,400	14,700
Foundry and shell moldings	39,000	43,600	50,000
Insulating materials	88,000	107,000	112,000
Laminating			
Building	31,500	26,100	26,200
Electrical/electronics	7,000	7,300	7,300
Furniture	12,000	16,000	17,000
Plywood	152,000	163,000	125,000
Protective coatings	10,000	9,600	10,100
Molding compounds			
Appliances	17,500	31,800	41,400
Business machines	4,400	6,100	6,800
Closures	9,100	4,500	4,100
Electrical controls and switches	40,400	56,000	61,000
Telephones	9,800	9,300	9,500
Wiring devices	15,600	15,900	16,300
Housewares			
Utensils and handles	11,200	14,300	14,700
Machine parts	4,000	4,800	5,100
Transportation	--	27,700	30,270

A very successful use for the vinal (PVA) fibers is in injection molded rail-tie retainers in Japan. PVA polyester laminate is also being used in greenhouse glazing. PVA cloth and mat are used as surfacing veils to improve impact strength, weatherability, and abrasion resistance.

The major uses for the vinal fibers in the United States are found in the manufacture of chemical lace and paper. Consideration is being given to using vinal fibers as tire cord material.

The estimated consumption of the vinal fibers in the United States is 226,000 lbs. annually, all of which is imported.

A. TRENDS

In 1973 urea and melamine rose 13%, a drop of 2% from the previous year's growth rate, principally due to two factors: demand for plywood, a market for urea bonding, fell considerably, which resulted in a urea-in-plywood total over 1000 tons lower than in the year before. The short supply of phenolics (purchasers turned to urea) kept the figures from becoming even lower. Expected large growth in melamine molding powders for dinnerware did not materialize; consumption was at the 1972 level.

Bonding and adhesive resins for fibrous and granulated wood have continued to be big market performers.

The acetals experienced a growth rate of 18.5% over 1972. Plumbing and machinery parts predominated. New uses included one-piece tape spools and aerosol containers.

CBS Records designed the one-piece tape spool for its Mark 2 eight-track tape cartridges to replace a two-piece polystyrene/acetal assembly with a one-piece part molded of Delrin (duPont) homopolymer.

The phenolics showed a gain of 0.4% over 1972. Their 11.3% increase in molding powders and advances in bonding and adhesive resins were offset by a major drop in the plywood sector. Molding powders were in

brisk demand for appliances, electrical/electronics, housewares and machine parts. Closures yielded to thermoplastics. Suppliers see a distinct trend to greater use for injection over transfer and compression. In other areas demand for phenolic resins for abrasive, friction, and foundry applications was up about 15%, reflecting activity by such major steel users as the automotive industry (Modern Plastics, 1974; 1).

Century growth figures have been projected (in metric tons) as: from 362,000 metric tons produced in 1971, the production rate of urea and melamine resins will reach 2,000,000 metric tons in the year 2000, with an annual growth rate of 6.1%.

The phenolic resins will increase in production from 541,000 metric tons in 1971 to 3,000,000 metric tons in the year 2000, with an annual growth rate of 6.1% (Modern Plastics, 1973; 7).

IV. CURRENT PRACTICE

There are no particular problems in the storage, transport, or handling of the formaldehyde resins; the completely polymerized finished resins are physiologically inert, non-toxic materials.

The problems lie in the manufacturing and in the disposal of these resins. Most of the starting reagents are toxic and must be carefully handled. The finished products contain nitrogen, and the combustion by-products produced by high-temperature incineration often are more noxious than the plasticizers. The combustion products are given in Section V, ENVIRONMENTAL CONTAMINATION.

V. ENVIRONMENTAL CONTAMINATION

Environmental contamination by the formaldehyde resins occurs both during manufacturing processes and during disposal processes.

While the completely polymerized finished plastics are dermatologically inert, most of the starting products are highly irritating to the skin and mucous membranes. Where careful precautions are not used, resin dusts easily contaminate the air in workshops and might possibly be carried from there into the atmosphere. In certain media, small amounts of formaldehyde can be released and can then oxidize and result in formic acid, a highly caustic and toxic compound.

Formaldehyde, cresylics, and phenols are present in the waste water from phenolic resin production. In the impregnation of paper with phenolic resin, low molecular weight resin is driven off during the impregnation and during the curing process in treater ovens. With less than perfect precautionary measures, both water pollution and air pollution will occur from these sources.

The thermo-oxidative destruction of the formaldehyde resins results in volatile toxic products. In the combustion of phenol-formaldehyde resins, water and formaldehyde are released at temperatures below 400°C; carbon monoxide, carbon dioxide, benzaldehyde, benzene, toluene, methane, phenol, and phenolic derivatives are released at temperatures between 400°C and 600°C; and carbon monoxide and hydrogen are released at temperatures above 600°C (Dotreppe-Grisard, 1968).

During the combustion of melamine-formaldehyde resins and urea-formaldehyde resins, the following products result: hydrogen, methane, acetylene, ethene, ethane, propylene, propane, butene, butane,

methyl alcohol, ethyl alcohol, acetone, acetic acid, furan, formaldehyde, methylfuran, dimethylfuran, benzene, toluene, carbon monoxide, carbon dioxide, ammonia, and cyanic acid (Hiramatsu, 1967).

An air purifying device for smokestack mounting has been developed by Marks Polarized Corp., Whitestone, N.Y. (U.S. Pats. 3,503,704 and 3,520,662). Polluted air is passed through an aerosol composed of charged water droplets. The device removes 99% of suspended particles, noxious gases, and other plastics combustion products (Anon., 1970; 1)

Three Japanese manufacturers have offered solutions to air pollution from plastics manufacture. Takuma Boiler Manufacturing Co. has designed an incinerator capable of burning several different types of plastics simultaneously, without emitting any polluting gases. This incinerator can handle 100 metric tons/day.

Okumura Kikai's model is smokeless within three minutes after firing. Forced compressed air allows the furnace to be fired without a starter. While a variety of plastics can be burned, only one type of resin can be destroyed in a single load.

Takuma's model burns pulverized plastics at 300°C. The gases given off are automatically passed into a second furnace and burned at 1000°C. Then the load is passed through a heat exchanger and then through a dust collector. The remaining waste is collected in a smokestack (Anon., 1970; 2).

A large processing line installed at the Narmco Materials Div. of Whittaker Corp., Anaheim, Calif. converts its own pollutants into a source of energy for heating and air conditioning. This company pro-

cesses glass fabrics impregnated with epoxy or phenolic adhesives. The thermosetting resin systems allow solvents to evaporate during heating. The fumes are drawn out of the oven and piped to the incinerator section of the heating system, where they are mixed with natural gas and burned. The intense heat of combustion produces steam in the boiler section of the system, and the steam is piped back to heat the air from the curing oven. These fumes provided 20% of the fuel required for process heat and plant air conditioning.

Emissions consist of carbon dioxide and fall within safety limits established by the Air Pollution Control District (Hauck, 1971, 1).

At the Spaulding Fibre Co., Tonawanda, N.Y., a closed-loop system is used to incinerate all wastes from plastics manufacturing. The resultant heat is then used to generate steam which powers the plant. Spaulding produces high-pressure laminates, basic phenolic resin, and paper (Anon. 1971; 1).

A molding machine that can process 100% of scrap regrind has been developed by Werner and Pfleiderer Corp., Waldwick, N.J. The machine (Remaker) will handle film scrap directly, without grinding or other intermediate conditioning. Virtually any type of thermoplastic reportedly can be molded by this method (Hauck, 1971; 2).

VI. MONITORING AND ANALYSIS

A. AMINO RESINS

The urea-formaldehyde resin content of paper can be measured in the 0.3% to 3% range by differential infrared spectrometry (Wise and Smith, 1967). Standards are prepared by adding a known volume

of standard solution to a paper of known weight and calculating the resin added.

Any contaminants present are removed from the paper test sample by successive extractions with carbon tetrachloride and methyl ethyl ketone, using a Soxhlet apparatus. The paper is then placed in a beaker of acidulated ethanol (4 cc of hydrochloric acid per liter), heated to just below the boiling point, and decanted after one-half hour. This process is repeated until the supernatant liquid is colorless. Hot water is used as a final extractant. The specimen is air-dried and then oven-dried at 105°C for one-half hour. This extraction procedure causes no detectable change in the urea-formaldehyde resin content of the paper, as measured at 6.05μ.

A 1:1 blend of polybromotrifluoroethylene and tetrachloroethylene is used as a coating liquid for both the standard sample and the test sample. The thickness of both of these specimens should correspond to 0.002 to 0.005 g/cm^3 to permit accurate measurement of the aliphatic carbon-hydrogen stretching band at 3.4μ (the internal standard). The coating liquid must be equal, in amount and in thickness, on both the sample and the standard specimens.

The specimens are mounted between sodium chloride plates. The spectrometer is operated at the highest programmed slit width of 1000 to provide a high signal/noise ratio.

Whenever the amount of urea resin is as high as 0.5%, it is possible to identify the resin by the presence of both major amide bands at 6.05 and 6.4μ.

Melamine resins in wet-strength papers can be detected and

estimated by ultraviolet spectrophotometry (Hirt, King, and Schmitt, 1954).

The cut-up paper samples are refluxed in 0.1 N hydrochloric acid to extract and hydrolyze the resin to the melaminium ion which is then measured spectrophotometrically. Melamine has a strong absorption near 235 mμ. This maximum, which is achieved in hydrochloric acid, becomes a slight shoulder in neutral or alkaline medium, thereby confirming the presence of melamine. If no band near 235 mμ is observed in hydrochloric acid, the presence of melamine can not be reported.

Braun and Jung (1970) present a simple method for differentiating between urea-formaldehyde and melamine-formaldehyde resins when both are present in a test resin sample. The test material is hydrolysed in concentrated hydrochloric acid by heating the mixture to the boiling point. An aliquot of the hydrolysate is made alkaline with dilute sodium hydroxide, and a drop of sodium hypochlorite is added. In the presence of a urea-formaldehyde condensate, the solution remains colorless at this point, while carbon dioxide is generated; a melamine-formaldehyde condensate gives a white precipitate. The precipitate is filtered and a drop of sodium hypochlorite is added. A yellow to orange color developing within thirty minutes is indicative of the presence of melamine.

To another aliquot of the hydrolysate, freshly prepared furfurol reagent is added (5 drops of pure, freshly distilled furfurol, 2 ml of acetone, 1 ml of concentrated hydrochloric acid, and 2 ml of water). The presence of a urea-formaldehyde condensate is indicated by the

development of a yellow to red color; the presence of melamine has no effect.

These same authors also describe a thin-layer chromatographic method for distinguishing between urea-formaldehyde and melamine-formaldehyde resins. The test sample is hydrolysed in 1 N sulfuric acid for two hours in a boiling water bath, after which any unhydrolysed portion is filtered out. The formaldehyde formed is distilled off until no further reaction is given between the distillate and carbazol/sulfuric acid. The pH of the test mixture is then adjusted to 6.5 with diluted barium hydroxide, and the precipitate is centrifuged out. Varying amounts of the solution, from 3.0 µL are applied to thin-layer plates, and the results are compared with control samples.

The following Rf values are given when 3 µL of test solution is applied, using a 15 cm path:

Solvents	Melamine	Urea
Pyridine:Benzene:Water	0.20	0.27
Acetonitrile:petroleum ether:carbon tetrachloride: tetrahydrofuran:formic acid:water (80:10:10:10:4: 10)	0.52	0.69
Acetonitrile:chloroform:benzene:methyl alcohol: water (20:50:30:40:10)	0.31	0.39
Pyridine:benzene:acetonitrole:water (50:50:30:10)	0.44	0.57
Pyridine:benzene:acetonitrile:water (50:50:30:5)	0.12	0.20
Pyridine:benzene:acetonitrile:water (50:80:60:5)	0.23	0.36
Acetonitrile	0.00	0.03

The free formaldehyde present in urea-formaldehyde foams can be determined by the method of Ardelt and Opel (1962). A foam sample, 10 x 10 x 5 cm, is crumbled under 1600 ml of water and kept for thirty

minutes. A 50 ml aliquot of the supernatant solution is transferred to a 100 ml volumetric flask containing 10 ml of N sodium hydroxide. The formaldehyde is then determined polarographically under nitrogen. The usual adjuncts of the foam do not interfere.

The formaldehyde present in textiles which have been treated with either urea-formaldehyde resin or malamine-formaldehyde resin can be determined by first extracting 36 cm^2 of material with 250 ml of water for 24 hours at room temperature. Two ml of sulfuric acid and a 0.5% soulution of the sodium salt of chromotropic acid is added to 2 ml of the extract. The mixture is then heated and held at a temperature of 100°C for 15 minutes. After 30 minutes, the absorbance is measured at 575 mμ. The precision is 2 μg (Vankos, Borza, and Palfi, 1967).

B. ACETAL RESINS

No analytical techniques for the acetal resins were found in the literature collected for this study.

C. PHENOLIC RESINS

The phenolic content of resins of unknown origin can be estimated by a modification of the nitrous acid test for free phenols, which produces a yellow color that is specific and is colorimetrically applicable (Swann and Weil, 1956).

A small sample of resin, varnish, or enamel vehicle is weighed, dissolved in n-butyl acetate, and diluted to definite volume. An aliquot of the resulting solution, estimated to contain not more than

6 mg of phenolic resin, is transferred to a 250 ml Erlenmeyer flask. Butyl acetate is added to bring the total volume to 40 ml. Ten ml of 10:1 sulfuric acid (3.6 N) is added, followed by 2 ml of a freshly prepared 10% aqueous solution of sodium nitrite. The flask is vented and placed in a 70°C water bath for one hour, during which time gentle agitation is applied. The sample is then cooled and transferred to a separatory funnel with water. The solvent layer is washed twice with water. After the final water layer is removed, the solvent layer is filtered into a 50 ml volumetric flask and diluted to volume. Colorimetric comparison is made at 425 mμ, against a blank cell of water. The phenolic resin content of the sample is determined from a calibration chart plotted from the results of tests on known standards.

A rapid gas-chromatographic method for determining phenol-formaldehyde resin in plywood adhesives was developed by Stevens and Percival (1964). A dual gas chromatograph is used. Column A is copper tubing, twelve feet in length and one-fourth of an inch in diameter, packed with silicone SF-96 (for phenol). Column B is copper tubing, sixteen feet in length and one-fourth of an inch in diameter, packed with 10% sucrose octoacetate on Teflon 6 (for formaldehyde). The operating conditions are: detection cell, 250°C; d.c. current, 200 ma; injection temperature, 250°C; column temperature, 130°C; and helium flow rate at 60 p.s.i.g., 120 ml per minute through column A and 59 ml per minute through column B.

The phenolic plywood adhesives can be diluted with water and injected directly into column B for formaldehyde analysis; this, however,

causes plugging of the injector and other mechanical difficulties. To preclude these difficulties, the resin solids are precipitated by neutralizing with acid and the aqueous solution is injected.

Ten grams of resin are weighed out, together with the internal standards: 1-butanol for formaldehyde and m-cresol for phenol. The sample is then diluted with approximately equal volumes of water and divided into two fractions. For formaldehyde analysis, one fraction is acidified with concentrated hydrochloric acid or sulfuric acid with vigorous stirring. The resin solids are then filtered off, leaving the aqueous solution for injection into column B. Ten ml of ether is added to the solid fraction, and the mixture is stirred vigorously while slowly adding acid. When neutralization is complete, the syringe is filled from the ether layer for injection into column A.

The peaks are read on a Leeds and Northrup Speedomax Type G Recorder.

The application of pyrolysis gas chromatography to the analysis of phenol-formaldehyde resins is reported by Zulaica and Guiochon (1966). The samples used (0.1-0.5 mg) were in the form most conveniently obtained: powder of resols or irregular fragments of resite.

The pyrolysis is carried out at temperatures between 700 and 750°C in a conventional platinum coil. The experiments are carried out on a Perkin Elmer 116 instrument under the following conditions: the column is nine meters long and four mm in diameter, packed with 4% tri(2,4-xylenyl)phosphate on Chromosorb P (250-315 μ). The working temperature is 180°C.

The pyrolysis products are detected on a katharometer.

VII. CHEMICAL REACTIVITY

The formaldehyde resins, infusible and chemically inert in the finished state, undergo no transformations under normal, use-related conditions.

The amino resins are intrinsically flame-resistant and have outstanding resistance to chemical solvents. The acetal resins are unusual among thermoplastics in their resistance to organic solvents, but they are subject to attack by strong acids and strong bases. The phenolic resins are considered to be nonburning materials which are unaffected by most chemicals.

However, the formaldehyde resins are organic materials and all organic materials will undergo combustion when the proper conditions are met. The main pyrolysis products of these resins are listed in Section V, ENVIRONMENTAL CONTAMINATION.

VIII. BIOLOGY

A. Absorption

The physical state of the finished formaldehyde resin products would preclude their entry into a living system. The starting products and the pyrolysis degradation products, however, are absorbable.

The primary candidate for absorption through the respiratory tract would be formaldehyde itself, a flammable, colorless gas at room temperature. Volkova and Sidorova (1971) found formaldehyde in the blood of 100 workers in an amino resins manufacturing plant. Eighteen hours after leaving the contaminated area, no formaldehyde was detected in the blood of these workers.

Although no cases of dust inhalation with sequela by workers are reported in the literature collected, this occurrance is a distinct possibility. Six months after a single inhalation of the dust of acetal resins, morphological changes were apparent in the lungs of experimental rats (Kochetkova, Vasil'eva, Promyslova, and Sergeev, 1971).

Carbon monoxide and carbon dioxide, both toxic, are the ultimate pyrolysis products of all plastics. The urea resins and the melamine resins give off ammonia as a degradation product. Since these three products are gases, they can be absorbed through the respiratory tract.

Accidental ingestion of formaldehyde resins would be unlikely. The Food and Drug Administration (Anon., 1964) has approved the use of melamine-formaldehyde resins for use in dinnerware. Since these resins are hard, mar-resistant, and resistant to oils, solvents, and greases, contamination of food from contact with these resins is most

improbable. Phenol-formaldehyde resins have been approved for use as a food-contact surface of molded articles intended for repeated use in contact with nonacid foods of pH above 5.0 (Anon., 1966).

B. EXCRETION/ELMINATION

Urea-formaldehyde resin and phenol-formaldehyde resin were excreted without effect, following the administration of 5 gm/kg into the digestive tracts of rabbits and rats (Galibin, 1963).

No acetal resin particles were found in the lungs of rats which had been subjected to resin-dust inhalation for twenty-nine days. It was assumed that the dust particles were caught in the mucous of the respiratory tract and were excreted when the mucous was discharged (Kopecny, Cerny, and Ambroz, 1968).

C. TRANSPORT AND DISTRIBUTION

Kopecny, Cerny, and Ambroz (1968) carried out toxicity studies on rats by administering the dust of acetal resins by inhalation, by subcutaneously injections, and by intraperitoneal injections. These authors did no transport and distribution studies, but stated that this work should be done, in reference to organs of deposition of the acetal powder following its injection into living systems.

D. METABOLISM AND METABOLIC EFFECTS

No metabolic studies were reported in the literature collected. However, the granuloma formation consequent to the injection of certain formaldehyde resins into experimental animals would indicate that these resins are not metabolized, but are phagocytized. Granulomas were found in the lungs of rats which had inhaled the dust of acetal resins (Kochetkova, Vasil'eva, Promyslova, and Sergeev, 1971) and granulomas

were found in the subcutaneous tissue and in the peritoneal region of rats after acetal resin powder had been injected at these sites (Kopecny, Cerny, and Ambroz, 1968).

IX. ENVIRONMENTAL TRANSPORT AND FATE

A. PERSISTENCE AND/OR DEGRADATION

Finished products constructed of formaldehyde resins persevere in the environment. They are flame-resistant, resistant to most chemicals, and resistant to wear. Therefore, they are not subject to degradation under normal, use-related conditions.

However, when the right conditions are present, these resins will undergo combustion, as will all organic materials. The resultant combustion products are listed in Section V, ENVIRONMENTAL CONTAMINATION.

B. ENVIRONMENTAL TRANSPORT

The tremendous utility of the formaldehyde resins would indicate the degree to which materials constructed from these resins are encountered daily.

Environmental concern, however, should be directed to the transport of the starting products, to the dusts formed during manufacturing, and to the decomposition products which are released on combustion of these resins. These subjects are discussed in Section V, ENVIRONMENTAL CONTAMINATION.

C. BIOACCUMULATION

Slensky and Horn (1971) studied a group of twenty-four workers who had been exposed to the dust released during the processing of 40% phenol-formaldehyde resin with 60% glass fiber. Some subjective

signs of the effect of this substance on the skin and respiratory mucosa were noted, but without signs of actual damage. These authors concluded that the short time-span of exposure of the workers (4-12 months) precluded definitive results, and stated that further work must be done.

The incidence of granuloma formation in experimental animals would indicate bioaccumulation of dusts and powders of formaldehyde resins. Granulomas were found in the lungs of rats who had been subjected to the inhalation of the dust of acetal resins. Granulomas were also found in the subcutaneous tissue and in the peritoneal region of rats after acetal powders had been injected at these cites (Kochetkova, Vasil'eva, Promyslova, and Sergeev, 1971; Kopecny, Cerny, and Ambroz, 1968).

X. TOXICITY

A. HUMAN TOXICITY

Every individual has daily contact with the myriad of products which are manufactured from the formaldehyde resins, yet there is no instance reported in the literature collected of any physiological reaction resulting from contact with a finished product. It is the contact between workers and the starting products used, the vapors and dusts formed during the manufacturing process, or those intermediate, unfinished products which might contain free formaldehyde or phenol that is the primary source of toxicity to humans from these resins.

Formaldehyde, a colorless gas which is intensely irritating to the mucosa and is a sensitizing agent, can be inhaled or can contact the skin directly in either the gaseous or the dissolved states. Phenol is a compound which is highly corrosive to the skin, and which, in

concentrated form, produces severe skin burns on contact. Urea and melamine are not toxic and the dermatitis encountered in the manufacture of resins containing these compounds is probably due to formaldehyde or to other components.

1. Inhalation

Formaldehyde was detected in the blood of 100 workers who were engaged in the manufacture of urea-formaldehyde resins. There was a direct correlation between the concentration of formaldehyde in the air and its level in the blood of the workers. The formaldehyde was detectable in the blood within fifteen to seventy minutes after the work began, and disappeared from the blood within eighteen hours after the workers left the contaminated areas. No toxic effects from this inhalation were reported (Volkova and Sidorova, 1971).

An increased incidence of illnesses was found among 103 workers in a plant producing asbestos-filled phenol-formaldehyde resin, in comparison with the incidence of illnesses among workers engaged in another type of production. The most common complaints were chest pains, headaches, and skin rashes. Chronic rhinitis was found in fourteen workers and pneumonia was discovered in three workers. These persons had been exposed for seven years to the vapors of phenol and of formaldehyde and to dust concentrations up to 132 mg/m^3 (Troitskii, Kuz'minykh, Andreeva, and Bunimovich, 1970).

2. Skin Contact

Dueva (1966) established a direct relationship between the sensitizing effects of urea-formaldehyde resins on the skin of workers and the amount of residual free formaldehyde contained in the resins.

Contact eczema attributable to melamine resin which had been incorporated into surgical bandages affected four nurses who had handled from two to fifteen bandages daily for a period of two weeks to five

months. One patient on whom this type of bandage had been applied also showed symptoms of eczema. After the skin eruptions had healed, cutaneous tests showed sensitivity to melamine resin, but no sensitivity to formaldehyde itself (Loechel, Lenz, and Herter, 1971).

Severe, vesicular, and exudative dermatitis occurred in six patients after receiving orthopedic casts which were reinforced with melamine-formaldehyde resin. The casts contained 10% of the melamine resin (0.01 to 0.3% of free formaldehyde). Patch tests proved that these patients were sensitive to formaldehyde (Logan and Perry, 1973).

Among forty-five workers who were exposed to bakelite powder (phenol-formaldehyde resin), 13% showed eczematous skin lesions accompanied by intesne itching in the areas in contact with the bakelite dust. The skin lesions were attributed to the resin dust in the air settling on exposed surfaces of skin. No symptoms from the inhalation of this dust are reported (Spalinska, 1971).

In a bakelite molding facility, twelve of the eighty persons employed contracted dermatitis within a four-year period. The lesions appeared as localized erythemotous papules. After the affected individuals were moved to work areas removed from the contaminated areas, the lesions regressed (Bresson, Bertholon, and Girard, 1972).

B. TOXICITY TO NON-HUMAN MAMMALS

1. Acute, Subacute, and Chronic Toxicity

The toxicity of urea-formaldehyde resin and phenol-formaldehyde resin was studied in rabbits and rats. Administration into the digestive tract of 5 gm/kg had no toxic effects. Cutaneous applications of these resins were also without sequel (Galibin, 1963).

Dust generated in the manufacturing of products consisting of 40% phenol-formaldehyde resin and 60% glass fiber was injected intratracheally into rats (75 mg/rat). After 11 months the larger amount of the dust had been eliminated. A negligible amount of small glass fiber fractions were found in the lungs in pulmonary phagocytes or in agglomerates surrounded by phagocytes. Inflammatory changes were seen in some bronchi. Collagen tissue formation was not induced (Sklensky and Horn, 1971).

Twenty rats were subjected to inhalations of acetal resin dust. The inhalations (approximately 5 gm per rat) were administered for thirty minutes daily, six days a week, for one month. During the course of the experiment, three animals perished: one from pneumonia, one from hemmorhage into the myocardium, and the third from undetermined causes.

Two months after the termination of the experiment, the rats were sacrificed by exsanguination. Upon comparison with the controls, there were no striking changes other than a slight increase in lymphoid tissue and edema of the adventitia of the larger pulmonary vessels. The adventitia were infiltrated by mononuclear and polymorphonuclear eosinophils.

An acetal resin suspension in 2 ml of saline was administered intraperitoneally to a group of five rats. Autopsy revealed small, whitish, hard and smooth granulomas attached to the base of the serous membranes, but no inflammatory changes. Microscopical resin particles were encapsulated in the granulomatous tissue.

In a group of five rats which received a saline suspension of acetal resin by subcutaneous injection into the area of the spinal column,

only a single resorbing granuloma was found.

No other changes were observed in the organs of the experimental animals--kidneys, spleen, liver, or myocardium (Kopecny, Cerny, and Ambroz, 1968).

Kochetkova, Vasil'eva, Promyslova, and Sergeev (1971) found phagocytes, granulomas, and inflammation in the lungs of rats of which had inhaled acetal resin dust. Morphological changes were apparent in the lungs six months after a single inhalation.

2. Sensitization

None of the animal studies in the literature collected reported sensitization reactions to the formaldehyde resins.

3. Teratogenicity

No teratological effects from the formaldehyde resins is reported in the literature collected.

4. Carcinogenicity

No malignant growths attributable to the formaldehyde resins or to their constituents are reported in the collected literature.

However, granuloma formation was induced in the lungs of rats which had been subjected to the inhalation of acetal resin dusts and granulomas were found in the peritoneal and subcutaneous areas of experimental animals at the sites of injections of saline suspensions of acetal resin dusts (Kochetkova, Vasil'eva, Promyslova, and Sergeev, 1971; Kopecny, Cerny, and Ambroz, 1968).

5. Mutagenicity

No studies relating the formaldehyde resins to mutagenicity are reported in the available literature.

6. Behavioral Effects

There are no reports in the collected literature concerning any behavioral effects of the formaldehyde resins.

C. TOXICITY TO LOWER ANIMALS

No studies were encountered concerning the toxicity of the formaldehyde resins toward lower animals.

D. TOXICITY TO PLANTS

No reports were found reporting the toxicity of formaldehyde resins toward plants.

E. TOXICITY TO MICROORGANISMS

Formaldehyde has had widespread use as a tissue preservative and as a disinfectant. Phenol is a known bacteriocidal agent and, in fact, is used as a standard of comparison in measuring the effectiveness of other antiseptics.

Although no studies appear in the collected literature concerning the toxicity of the formaldehyde resins to microorganisms, any residual free formaldehyde or phenol present in the resins would have a bacteriocidal effect.

XI. CURRENT REGULATIONS

A. Food and Drug Administration

According to the Food and Drug Administration (Anon., 1964), formaldehyde resins may be safely used as a food-contact surface on molded articles which are used in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food. For these purposes, melamine-formaldehyde resins are defined as the reaction products of one mole of melamine and not more than three moles of formaldehyde in aqueous solution. The molded melamine-formaldehyde articles in the finished form (in which they are to contact food) must not yield chloroform-soluble extracts in excess of 0.5 mg/sq. in. of food-contact surface.

Phenol-formaldehyde resins may also be used under the Federal Food, Drug, and Cosmetic Act as the food-contact surface of molded articles (Anon., 1966). This rule applies to repeated contact with nonacid food (pH > 5.0), if the finished article meets the following specifications: when extracted with distilled water at reflux temperatures for two hours, using a volume-surface ratio of 2 ml of water to 1 sq. in. of surface, the total extractives should not exceed 0.15 mg/sq. in.; the maximum phenol detection is 0.005 mg/sq. inc., with no extracted aniline. These determinations should be run by a spectrophotometric method sensitive to 0.006 mg/sq. in.

B. The Occupational Safety and Health Act

The Occupational Safety and Health Act of April 28, 1971, has special significance for the plastics industry, since this industry has such a poor safety record. The act states that each employer "shall

furnish to each of his employees, employment and a place of employment, which are free from recognized hazards that are causing, or are likely to cause, death or serious physiological harm to his employees".

This bad safety record is a matter of accidents with the equipment, however, and not a matter of hazard from contaminated areas (Anon., 1971; 2).

Other OSHA regulations are discussed in Section XII, STANDARDS.

C. Department of Transportation

The Department of Transportation has issued flammability rules for automotive interiors and has proposed upgrading regulations covering passenger airplane furnishings (Anon., 1972; 2). The formaldehyde resins would be included in the plastics which would be under consideration here.

Test methods for special materials for aircraft applications will be established by ASTM Committee F-7. Tests will be developed to evaluate safe performance of cleaners for plastics and other exterior and interior surfaces (Anon., 1973).

D. Air and Water Acts

Acetal copolymer has been approved by the National Science Foundation for use in contact with water to be used for drinking (*Modern Plastics Encyclopedia*, 1970-71, pg. 88).

E. State, Federal, and Foreign Regulations

A New York City tax on plastic containers was declared unconstitutional on November 11, 1971. Justice Saul S. Streit of the Supreme Court of New York State ruled that such discrimination against plastics was arbitrary and unreasonable, and thus a violation of the equal protection clauses of the Federal and New York State Constitutions. Justice Streit stated, "There was not one shred of evidence presented herein which demonstrates that any form of container, glass, metal, or paperboard, is any more

recyclable than plastic containers .... It thus appears that the discrimination against plastic containers does not rest on any reasonable basis in relation to the objective of promoting the recycling of containers, plastics or otherwise" (Kestler, 1971; Anon., 1972; 3).

Some further regulations are discussed in Section XII, STANDARDS.

XII. STANDARDS

A. Threshold Limit Values

In June, 1972, a federal regulation was published under the Occupational Safety and Health Act (OSHA), limiting the concentration of asbestos fibers (a filler for formaldehyde resins) in work areas to 5 units per cc of air, which is to be reduced to 2 units per cc of air by July 1, 1976. The previous threshold limit value for asbestos, published by the American Conference of Government Industrial Hygienists (ACGIH) was a limit of 12 units per cc of air.

To obviate investments in protective clothing, improved dust-collection and ventilation systems, and periodic examinations of workers, General Electric has complied with OSHA's ruling by changing its phenolic compound formulation. The identity of the substitute filler has not been revealed, but GE has said that it has been cleared by the Food and Drug Administration (Anon., 1972; 1).

The following figures show the maximum permitted atmospheric concentrations of formaldehyde and of phenol (in ppm and mg/m^3) in factories in Great Britain, the United States, and Russia.

COUNTRY	FORMALDEHYDE		PHENOL	
	ppm	mg/m^3	ppm	mg/m^3
Great Britain	-	12	-	-
United States	5	6	5	19
U.S.S.R.	-	1	-	5

B. Flammability

The National Materials Advisory Board, which evaluates materials on request from federal agencies, has begun a three-year study to gather, correlate, and analyze material on fire testing and fire behavior of plastics. The ultimate goal is to develop a model test that will duplicate any fire-situation performance. A twenty-member committee on the Fire Safety Aspects of Polymeric Materials has been formed.

The Fire Retardant Chemicals Association, formed in 1973, has launched an organized inquiry into the fire safety of polymerics. The aims of this new group include better communication with government agencies, and interaction with industry groups (Macbride, 1973).

The Underwriters' Laboratories are developing systems for plastic product safety. The UL temperature index (Reymers, 1970; a) correlates numerically with the temperature rating (the maximum temperature in °C above which a material may degrade prematurely and, therefore, be unsafe). The formaldehyde resins have been given the following temperature indices:

Urea-formaldehyde resins	100°C
Molded melamine resins (excluding fiber-reinforced)	130°C
Molded phenolic resins (excluding fiber-reinforced)	150°C

These figures apply to heat and pressure molded resins only; they do not apply to those resins intended for casting or pouring (Reymers, 1970; a).

Among the Underwriters' Laboratories flammability indexing tests are the self-extinguishing tests (the phenol-formaldehyde resins hold a Group I rating), the slow-burning tests, the hot-wire ignition tests, the high-current-arc ignition tests, the high-voltage-arc ignition tests, and the high-voltage-arc track tests.

Prior to 1973, the classifications were defined as follows:

SE-0	"Self-extinguishing, Group 0"	Do not release flaming particles and do not continue to flame longer than 10 seconds.
SE-1	Group I	Do not release flaming particles or drops.
SE-2	Group II	Release flaming particles or drops which burn only briefly.

(Reymers, 1970; b)

In September, 1973, Underwriters' Laboratories distributed a revised edition of its UL-94 test for flammability of plastics materials. The terms "SE" (self-extinguishing) and "SB" (slow-burning) have been deleted. "SE" designations have been replaced by the letter code "VE" (vertical flame extinguishing tests) and "SB" designations have been replaced by "HB" (horizontal burn tests). The testing parameters remain unchanged.

The American Society for Testing and Materials is in the process of revising the terminology of its D-635 flammability test. All references to the "NB" classification (nonburning by this test) are eliminated. The text contains a warning that the indicated properties are based on small-scale laboratory tests, and in no case are to be used as an assessment of actual fire hazards (Anon., 1973; 1).

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o-NITROCHLOROBENZENE

SUMMARY AND CONCLUSION

Since the only use for the material seems to be for conversion to dinitrochlorobenzene, it is unlikely that any more is released to the environment than that which comes from waste waters of production and conversion plants. This is fortunate because the limited data available indicate very high toxicity and very low biodegradability. Partial metabolism appears only to convert it to other toxic materials such as chloroaniline. Assuming that proper safeguards are observed in the industries involved, the only likely hazard is to life in and dependent upon bodies of water receiving the industrial waste, especially, in view of the density and water insolubility, bottom dwellers.

Production figures for o-nitrochlorobenzene were not published after 1967, but the 1963-1967 period was one of rapid increase. If the proportion of sales to production was maintained after 1967, then production continued to rise through 1969 (the last year for which sales figures are available). As of 1972 imports were not significant.

O-NITROCHLOROBENZENE

I. PHYSICAL PROPERTIES

This compound is a crystalline solid having (°C): mp 32-3, bp 245-6, flash point 127, d 1.368(22/4); it is soluble in benzene, ethanol, and ether, insoluble in water (0.44 g/l at 20°).

II. PRODUCTION

Table I. Production-Importation of o-Nitrochlorobenzene

Year	Made in USA[a,b,c]	Imported into USA[a]
1963	8,570 (1,779)	
1964	9,118 (-----)	
1965	12,841 (-----)	
1966	16,443 (-----)	
1967	15,535	
1968	--	
1969	--	
1970	--	
1971	--	0.12
1972	--	56

a - units are metric tons

b - figure in ( ) is for a mixture of o- and p- isomers

c - amounts sold were: 4,864; 4,379; 4,782; 5,590; 5,629; 6,637; 9,255; --; --; --

Currently the only known domestic producers are American Aniline Products, duPont, and Monsanto. There is no indication that the o/p mixture has been produced since 1966.

III. USES

The only known use is as an intermediate for dye synthesis, being converted to 2,4-dinitrochlorobenzene.

IV. CURRENT PRACTICE

Sax indicates that the IATA regulations for liquid nitrochlorobenzene require the container to bear a poison label, also the Poison B label; quantities are limited to one liter on a passenger craft, 220 liters on a cargo plane.

No information bearing on disposal methods was found.

V. ENVIRONMENTAL CONTAMINATION

No references were found which indicated the occurrence of this compound in the environment.

VI. MONITORING AND ANALYSIS

Piotrowski (1965) reported a colorimetric method for measuring chloronitrobenzenes in air. Dyatlovitskaya and Potemkina (1963) reported a colorimetric/polarographic method for distinguishing between chloronitrobenzenes and nitrobenzene present together. Fleszar (1964) reported an extraction/polarographic method for measuring 5-50 ppm of nitrochlorobenzenes in water. Kolbasov et al (1962) discussed an infrared technique for measuring the various nitrochloro-, 2,5-dichloronitro-, and 3,5-dichloronitrobenzenes present in a mixture. Stanescu and Radulescu (1970) reported an infrared method for analyzing a mixture of the ortho and para isomers ranging from 4:1-1:4.

Hashimoto et al (1965) determined R_f values in five solvents using thin layer chromatography (TLC). Obruba and Navratil (1967) used TLC to separate nitration mixtures of chlorobenzene consisting of the ortho and para mononitros, di- and trinitros.

Gas liquid chromatography is the favorite analytical tool for nitrochlorobenzenes and has been discussed in the following papers: Bykova *et al* (1969), dimethylsiloxane on diatomite, katharometer; Habboush and Norman (1962), dinonyl phthalate, tritolyl phosphate, trinitrofluorenone on Embacel, flame ionization detector; Habboush and Tameesh (1970), polyethyleneglycol 1500, polyethyleneglycol succinate on Chromosorb P, thermal conductivity detector; Nemova *et al* (1969), polyethyleneglycol 1000 on INZ-500; Roseira (1970), involved initial conversion to nitrobutylbenzene; Stolyarova *et al* (1965), polyethyleneglycol 1000 on brick; Volkova *et al* (1972), polyethyleneglycol 1500 on Celite 545, flame ionization detector; Zielinski *et al* (1967), trifluoropropylmethyl silicone on Chromosorb G, electron capture detector.

VII. CHEMICAL REACTIVITY

No information bearing on this matter was found.

VIII. BIOLOGY

A. Metabolic Effects

Bray *et al* (1956) compared the metabolism of the three nitrochlorobenzene isomers in rabbits. The dose of the ortho isomer given was a single one of 0.1 g/kg, and was the largest which elicited no signs of toxicity; in comparison, 0.2 g/kg doses of the other two isomers were still non-toxic. Urine and feces were analyzed for up to 48 hours after administration of the dose, by which time no more metabolites were present.

It was possible to account for 82% of the dose: 42% in the urine as a glucuronide; 24% in the urine as a sulfate; 9% in the urine and 0.3% in the feces as free o-chloroaniline; 7% in the urine as a mercapturate

(the values for the 42, 24, and 7% conjugates are medians of three animals; the urinary free chloroaniline was a pooled value of six animals).

The following free phenols (totalling 5% of the dose) were found in the urine in substantial quantities: 3-amino-4-chloro-, 4-amino-3-chloro-, 3-chloro-4-nitro-, and in trace quantities: 2-chloro-3-nitro-, 4-chloro-3-nitro-, 3-chloro-2-nitro-, and 3-amino-2-chloro-. The mercapturate found was the 2-nitro-phenyl. The following phenols were present as the glucuronide or sulfate conjugates in substantial quantities: 3-chloro-4-nitro-, 3-amino-4-chloro-, and 4-amino-3-chloro-, and in trace quantities: 2-chloro-3-nitro-, 2-amino-3-chloro-, and 3-amino-2-chloro-.

B. Physiological Effects

Shirai (1953) reported that factory workers exposed to nitrochlorobenzene had higher blood glutathione levels than normal people even when not showing any toxic symptoms; seasonal variation of this level was normal in the workers.

Frenkel and Gordienko (1960) found that by poisoning rats with nitrochlorobenzene the following changes in brain tissue chemistry occurred: preformed ammonia and glutamine levels increased; protein amino N fell by 15 mg%; none in levels of nonprotein N, glycogen, or ATP.

Frenkel (1963) reported results similar to those of the 1960 paper, adding that creatine phosphate level increased, and hypothesizing that these changes result from inhibition of anabolic synthetic reactions.

IX. ENVIRONMENTAL EFFECTS

A. Persistence and/or Degradation

Ludzack and Ettinger (1963) found that no more than 20% of the

theoretical amount of carbon dioxide was eventually produced from the degradation of o-nitrochlorobenzene in a sample of river water over an 80 day test period. In comparison, 50% of the theoretical amount of CO_2 from acetophenone evolved in five days.

Alexander and Lustigman (1966) found that there was no significant splitting apart of the ring by soil micro-organisms in a 64 day test period, indicating that the chemical was certainly not a food source for them. Determination of lesser degrees of metabolism or degradation was beyond the scope of their experiment.

B. Environmental Transport

C. Bioaccumulation

No information was found on either of these considerations.

X. TOXICITY

A. Human

Lutowiecki (1960) reported on a test for skin sensitivity in new workers, but the results are difficult to obtain.

Sax reported that acute or chronic local toxicity was unknown, but acute or chronic systemic toxicity from either ingestion or inhalation was high. The pathology was methemoglobin formation, cyanosis, and other blood changes; the effects were analogous with nitrobenzene, and were cumulative. Industrially, contact is most likely to be made from dust.

B. Birds and Animals

Bray *et al* (1956) reported that a single dose to rabbits of over 0.1 g/kg was toxic enough to interfere with their metabolism study (see VIII, A.), but didn't further elaborate.

Navrotskii (1953) reported that the production of methemoglobin following nitrochlorobenzene poisoning in animals was not affected by administration of adrenaline, but was initially depressed by acetylcholine, and blocked during the sleep induced by chloral hydrate or Medinal. When the narcotic effect of the latter two ceased and the animal awoke, methemoglobin production commenced.

Rusakov *et al* (1973) found that 8 µg/cu m was the minimum atmospheric concentration which would elicit allergenic sensitization in rats and guinea pigs. Transfer of serum from sensitized rats to guinea pigs resulted in allergic symptoms in the latter.

C. Lower Animals

No information on toxicity to this class of life was found.

D. Plants

Eckert (1962) found that 50% reductions in growth of cucumber and mung bean seedlings resulted from contact with 115 and 190 µM solutions, respectively, for six days.

E. Micro-organisms

Eckert (1962) found that 50% reductions in the propagation of the soil fungi Rhizoctonia solani and Pythium ultimum resulted from contact with 310 and 1,000 µM solutions, respectively.

Richardson (1968) found that the vapor phase toxicity: to P. ultimum was lower for the nitrochloro below 250 ppm (the compound being dispersed in soil at various concentrations, and the fungi being suspended above the soil); to R. solani was about the same as for nitrobenzene; to the saprophytic fungus Trichoderma viride was a bit lower than nitrobenzene.

Romanova and Rapoport (1971) reported a reduced viability of spores of Actinomyces sphaeroides after two-hour suspension in millimolar nitrochlorobenzene.

XI. CURRENT REGULATIONS

XII. STANDARDS

No information was found other than the IATA requirement mentioned in Section IV of this report.

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16. ABSTRACT

A review of the literature published from 1953 through 1973 was conducted to prepare this report on the physical and chemical properties of azo compounds, brominated hydrocarbons, EDTA, formaldehyde resins and o-nitrochlorobenzene, on environmental exposure factors related their consumption and use, on the health and environmental effects resulting from exposure to these substances and on any applicable regulations and standards governing their use.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
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