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Environmental Health Effects Research Series

TERATOLOGY AND ACUTE TOXICOLOGY OF SELECTED CHEMICAL PESTICIDES ADMINISTERED BY INHALATION

Health Effects Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

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TERATOLOGY AND ACUTE TOXICOLOGY OF SELECTED CHEMICAL PESTICIDES ADMINISTERED BY INHALATION

by

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory develops and revises air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

As part of its overall mission, the U.S. Environmental Protection Agency is concerned with the effects of pesticides on mammals including man. One area of specific concern is the effects of such compounds on the developing mammalian organism. The following report deals with the potential of selected pesticides to induce toxic effects in embryos and/or fetuses of animals exposed to such chemicals by the pulmonary route during gestation.

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PREFACE

The Federal Insecticide, Fungicide, and Rodenticide Act designates the Environmental Protection Agency as the governmental body responsible for the safety of all pesticides used in the United States. More recently, the Federal Environmental Pesticide Control Act (PL 92-516) strengthened EPA's regulatory responsibilities in the area of pesticides to include intra- as well as inter-state commerce.

To be federally registered, a pesticide must be determined to not be hazardous to health or to the environment when used according to its labeling restrictions. Thus, relative to the new law as well as to specific directives included in Public Law 93-135, 1973, EPA now is conducting a thorough review of the implications of using alternate chemicals for pest control, including older registered pesticides. Stanford Research Institute is contributing to these goals through two distinct programs engaged in

- comparative toxicity of pesticides administered by various routes, and
- teratology of inhaled chemicals.

This report includes the results of both studies mentioned above. The first compares the acute toxicity of Parathion, Methyl Parathion, Guthion, Azodrin, and Thimet when administered to male and female rats by the percutaneous, oral, intravenous, and inhalation routes. The second deals with the potential fetotoxicity of chloroform, ethylene thiourea, Thimet, Bromacil, and Simazine administered by daily inhalation on days 7 through 14 of gestation in rats. The results and discussion of both studies are dealt with separately in the following report.

ABSTRACT

A method was developed for generating pesticide aerosols within the respirable particle size range of 0.3 to 3.0 μm. Analytical methods were established for determining pesticide concentrations in chamber air samples and in tissues. A unique chamber exposure system was developed that permitted the simultaneous exposure of four different groups of rats to four different concentrations of pesticide from a single generation source. Parathion, methyl parathion, Thimet, Guthion, and Azodrin were administered to rats by the oral, dermal, intravenous or inhalation routes, and the LD_{50}s or LC_{50}s were compared. Inhalation was the most toxic route of administration, followed by the intravenous, oral, and then dermal routes. Females were more sensitive than males to parathion and Thimet by all routes of administration. Azodrin was more toxic to females by the intravenous and oral routes, and Guthion was more toxic to females by dermal application. No correlation was found between mortality and cholinesterase inhibition or blood or liver pesticide content. No gross or histopathological lesions were identified that could be attributed to pesticide treatment.

Timed-pregnant rats were exposed to vapors/aerosols of chloroform, ethylene thiourea, Thimet, Bromacil, and Simazine for 1 to 3 hours daily on days 7 through 14 of gestation. Three different concentrations of each compound were used for each study. All animals were sacrificed on day 20 of gestation and examined for total litter size, fetal weight, and fetal resorptions. No dose-related terata were found in any of the studies. Chloroform produced an increased embryotoxicity at 20 mg/liter (4100 ppm). The highest doses of ethylene thiourea and Thimet produced a small increase in resorptions.

This report was submitted in fulfillment of Contract No. 68-02-1751 by Stanford Research Institute under the Sponsorship of the U.S. Environmental Protection Agency.

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INTRODUCTION

The Environmental Protection Agency is conducting a thorough investigation of insecticides to determine their effects on health and the environment. Through a review of the literature and laboratory studies, the Agency anticipates that alternative chemicals for pest control may be recommended.

To support these aims, EPA commissioned the Toxicology Department of SRI to assess the acute single-dose toxicity of potent organophosphate pesticides preliminary to a comparative evaluation of candidate alternative or substitute agents and to study the fetotoxic potential of specified pesticides or herbicides to animals after inhalation exposure. In the first study, we compared the LD_{50} and LC_{50} values of parathion, methyl parathion, Thimet, Azodrin, and Guthion administered by the oral, intravenous, percutaneous, and respiratory routes. Also recorded were pesticide blood concentrations after exposure and/or blood cholinesterase values as well as gross and histopathologic changes.

This study was divided into the following five phases: (1) the generation of respirable particles; (2) development of analytical methods to determine the aerosol concentration in the exposure chamber; (3) acute toxicity study in rats; (4) analysis of animal tissue for insecticide content; and (5) examination of gross and histopathological changes of animal organs.

The fetotoxicity studies were divided into three phases. Pregnant rats were subjected to:

(1) Inhalation exposure to chloroform to reproduce results reported by Schwetz et al.[1]

(2) Inhalation exposure to ethylene thiourea, a potent teratogen when given orally in high doses,[2] to determine its teratogenicity when given by inhalation and to verify the methodology.

(3) Inhalation exposure to the insecticide Thimet and the herbicides Simazine and Bromacil to test their teratogenic potential.

All inhalation exposures were conducted at SRI. Fetuses were shipped to the Project Monitor at Research Triangle Park, North Carolina, for teratogenic evaluation.

This report describes the inhalation exposures and the observed effects on the rats and fetal changes in their offspring that had occurred by day 20 of gestation, when the animals were sacrificed.

MATERIALS AND METHODS

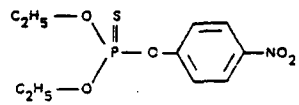
Animals

Adult male and female Sprague-Dawley rats weighing between 200 and 250 g were obtained from Simonsen Laboratories, Gilroy, California. The animals were housed in plastic cages with hardwood chip bedding and were provided with Purina Laboratory Rat Chow and water *ad libitum*. The animals were isolated upon arrival at the Laboratory and were allowed to acclimatize before use. All animals were observed closely during this time to ensure that only healthy animals were used for the subsequent studies.

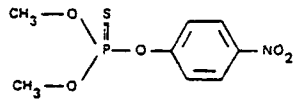
Pesticides

The pesticides selected for the general toxicity studies were parathion, methyl parathion, Thimet, Guthion, and Azodrin. Figure 1 presents their structural formulae, trade names, and chemical names. All the pesticides were supplied as technical-grade material from the Battelle Memorial Institute Repository, Columbus Laboratories, Columbus, Ohio.

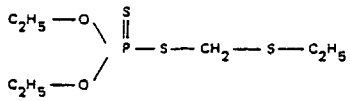
The chemicals selected for the fetotoxicity study were chloroform, ethylene thiourea, Thimet, Bromacil, and Simazine. Chloroform (analytical grade) and xylene were obtained from Mallinckrodt Chemical Works, St. Louis, Missouri. DMSO (dimethyl sulfoxide) was obtained from Crown Zellerbach, Camas, Washington. Ethylene thiourea (2-imidazolidinethione), practical grade, was obtained from Matheson, Coleman and Bell, East Rutherford, New Jersey. Thimet, Bromacil, and Simazine were obtained from the EPA pesticide repository at Battelle Memorial Institute, Columbus, Ohio.



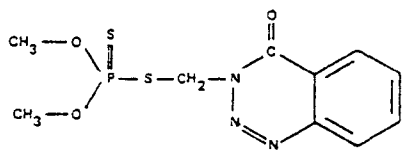
PARATHION: O,O–DIETHYL O–p–NITROPHENYL PHOSPHOROTHIOATE



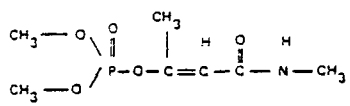
METHYL PARATHION: O, O–DIMETHYL O–p–NITROPHENYL PHOSPHOROTHIOATE



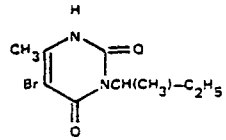
THIMET: O, O–DIETHYL S–(ETHYLTHIO) METHYL PHOSPHORODITHIOATE



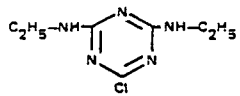
GUTHION: O, O –DIMETHYLS – (4–OXO–1,2,3–BENZOTRIAZIN–3 (4H) –YLMETHYL) PHOSPHORODITHIOATE



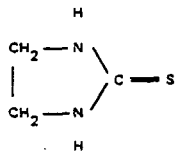
AZODRIN: cis–3–(DIMETHOXY PHOSPHINYLOXY)–N–METHYLCROTONAMIDE



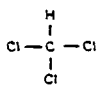
BROMACIL; 5–BROMO–3–SEC–BUTYL–6–METHYLURACIL(8CI)



SIMAZINE; 6–CHLORO–N,N–DIETHYL–1,3,5–TRIAZINE–2,4–DIAMINE(9CI)



ETHYLENETHIOUREA



CHLOROFORM

FIGURE 1. THE STRUCTURAL FORMULAE, TRADE NAMES, AND THE U.S. NOMENCLATURE OF THE NINE CHEMICALS STUDIED

Administration of Compounds

In the general toxicology studies, compounds were administered intravenously via the tail vein, orally via an intragastric tube, dermally on an area of the back that had been clipped free of hair, or by inhalation. Dilutions of compounds for intravenous, oral, or dermal application were made with propylene glycol. Technical-grade compounds were used for inhalation without being diluted.

In the fetotoxicity studies, all compounds were administered daily during day 7 through day 14 of gestation by inhalation using the methodology, inhalation chambers, and equipment described herein. Daily exposure durations were 1 hour for chloroform and Thimet, 2 hours for Bromacil and Simazine, and 3 hours for ethylene thiourea.

Aerosol Generation

Respirable aerosols were generated with either an ultrasonic or a pneumatic generator. As the aerosols were generated, the particulates were swept into a dilution chamber with intake air so that only a narrow range of particle sizes were carried into the aerosol distribution system. Aerosol concentrations needed for each chamber were obtained by regulating the exhaust and dilution air. Figure 2 is a schematic of the aerosol generation and distribution system. As the aerosol passed through the chamber, it was exhausted through a metering orifice that controlled the ratio of the concentrations between chambers. The exhaust was collected in a manifold and passed through a vacuum pump that maintained a negative pressure of about 0.5 inches of water in the chambers. The exhaust passed through an absolute filter and then through an incinerator, which burned at approximately 1100°C. The exhaust was then routed through an exhaust ejector and out the stack. Figure 3 is a schematic diagram of the arrangement of the entire exposure facility.

Chloroform vapor was generated by passing a small part of the chamber air intake over a chloroform surface in a 250-ml side-arm flask that was temperature-stabilized with a water bath. The air into the flask was regulated with a critical orifice so that flows were kept constant.

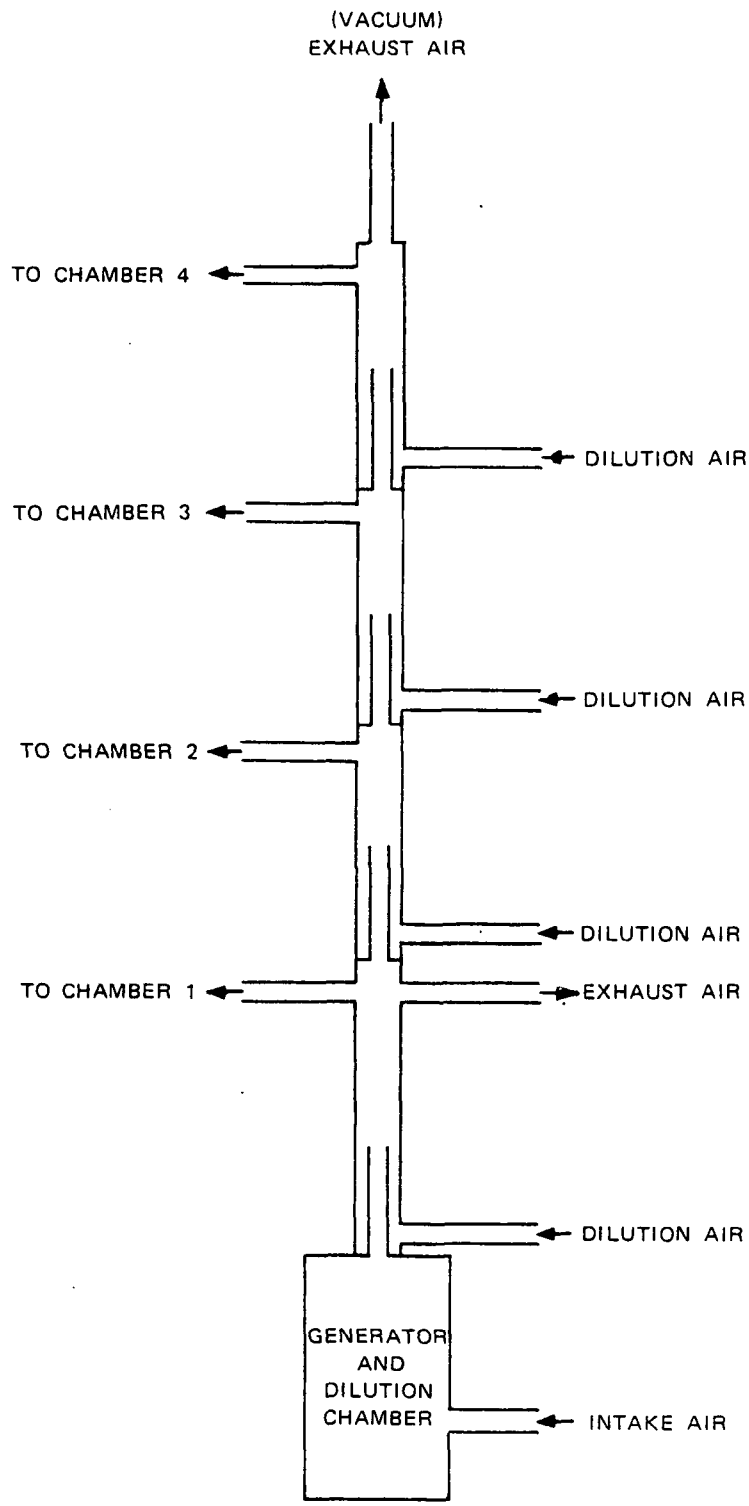


FIGURE 2 ARRANGEMENT OF THE AEROSOL GENERATION AND DISTRIBUTION SYSTEM

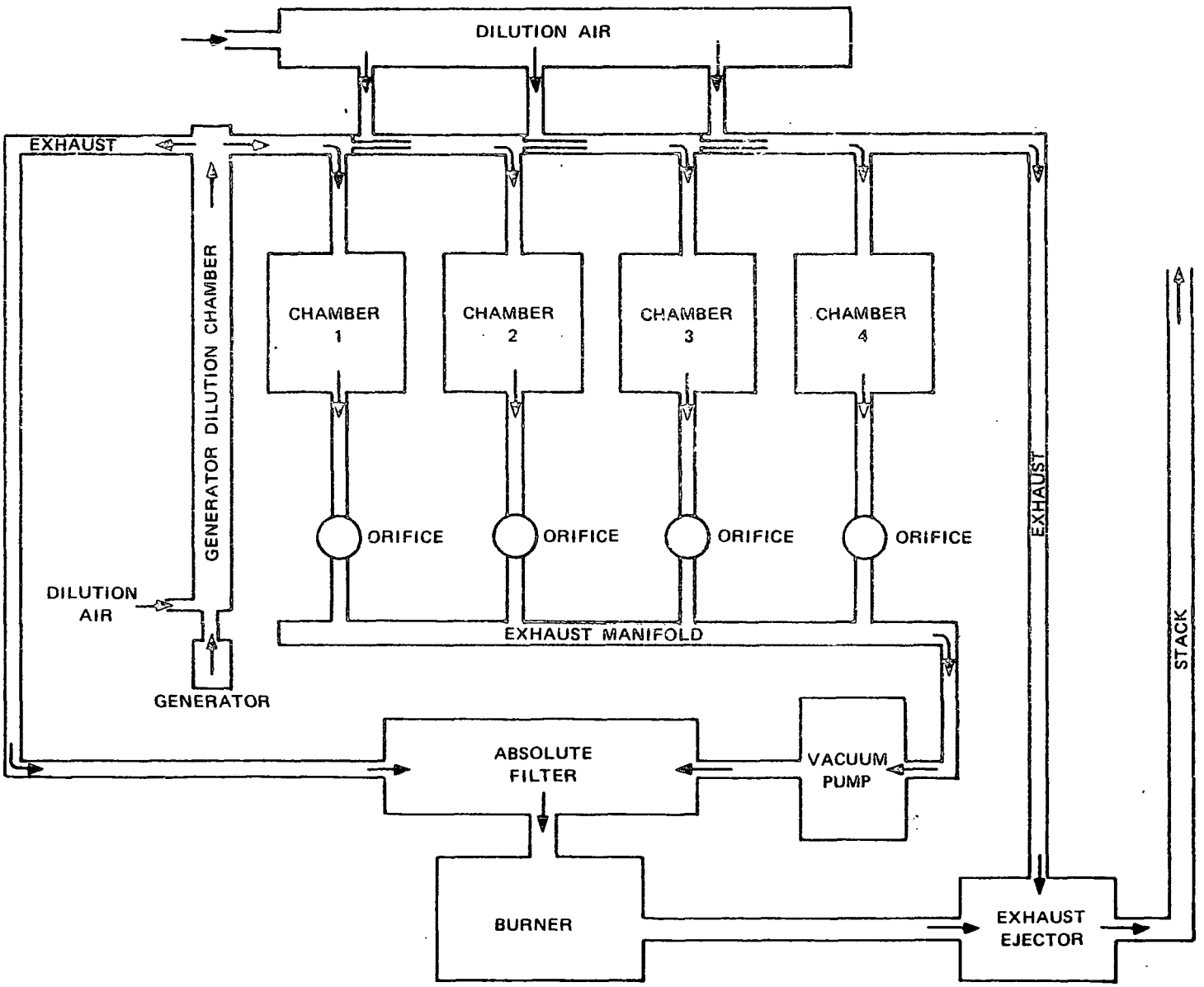


FIGURE 3 ARRANGEMENT OF THE AEROSOL EXPOSURE EQUIPMENT

Thimet aerosols were generated from a 1% solution of Thimet in xylene, using a pneumatic aerosol generator. The dilution of Thimet was necessary because of its acute toxicity (11 mg/m^3 for a 1-hour exposure in female rats) and because we desired to have some survivors after 8 days of daily exposure to the highest dose of the aerosols.

Bromacil, Simazine, and ethylene thiourea aerosols were generated with four DeVilbiss nebulizers operated at 15 psi. Water was used as the solvent for ethylene thiourea, but DMSO was used for both Bromacil and Simazine. We chose this method of generation because of a need to obtain high concentrations of aerosol in the exposure chambers.

Chamber Atmosphere Analysis

Chloroform was collected from the animal breathing area in the chambers by means of polyethylene PE-50 tubing and transferred into a 1.0-ml gas-tight syringe. This sample was injected directly into a Varian Model 1520 gas chromatograph equipped with a flame ionization detector. The column used was a 3/16" by 48" glass column packed with 3% OV-17 on Gas Chrom Q 100-120 mesh. Injector and column temperature were ambient and N_2 was the carrier gas.

Samples of all the other compounds tested were collected on nucleopore filters. A gravimetric determination was made on all samples with a Perkin-Elmer Model AD2 microbalance capable of weighing with an accuracy of ± 1 microgram. Gas chromatographic (GC) analysis of the material eluted from the filter was made with the Varian Model 1520 GC. For ethylene thiourea, we used a 3/16" by 48" glass column packed with 3% OV-17 on Gas Chrom WH; column temperature was 150°C and the retention time was 150 seconds. N_2 was used as the carrier gas. The detector was a photometric detector equipped with a sulfur filter.

The GC analysis for Thimet is described below.

Bromacil and Simazine were analyzed by GC using a flame ionization detector and a 3/16" by 48" glass column packed with 3% OV-17 on Gas Chrom Q. Methyl parathion was used as an internal standard for Bromacil, which had a retention time of 255 seconds at a column tempera-

ture of 210°C. Thimet was used as an internal standard for Simazine, which had a retention time of 755 seconds at the same column temperature.

Particle-Size Analysis

The particle sizes of the aerosols generated for these studies were detemined using a seven-stage cascade impactor. The particle sizes listed below are the mean and standard geometric deviation of the aerodynamic size (in microns).

Compound	Aerodynamic Mean (μ)	Standard Geometric Deviation (σg)
Thimet	0.44	2.50
Simazine	0.50	2.4
Bromacil	0.44	2.2
Ethylene thiourea	0.82	2.9

Pesticide Analysis

Pesticide analysis was performed with a Varian model 1520 gas chromatograph equipped with a flame photometric detector. The column used was 2" x 1/4" glass packed with 3% OV-17 on 80/100 mesh Gas Chrom Q. Detector flow rates were 20 ml/min O_2, 200 ml/min H_2; and 50 ml/min synthetic air. The carrier gas used was nitrogen; the carrier gas flow, the column temperature, and the solvent and internal standard used were different for each pesticide studied, as shown in Table 1.

Table 1

CONDITIONS FOR PESTICIDE ANALYSIS

Pesticide	Solvent	Column Temp. (°C)	N_2 Flow (ml/min)	Internal Standard
Methyl parathion	Acetone	200	60	Parathion
Parathion	Acetone	200	60	Methyl parathion
Thimet	Acetone	200	80	Methyl parathion
Azodrin	Acetone	225	60	Methyl parathion
Guthion	Benzene	245	110	7 Co-Ral

Pesticide samples were collected from each chamber on preweighed nucleopore filters. The filters were reweighed at the end of the exposure period to determine the total mass generated during the exposure. The pesticides were then eluted from the filter with a solvent and analyzed by gas chromatography. An Autolab 6300 digital integrator was used for analysis of peak area and retention time. The amount of pesticide on each filter was determined by comparison with an internal standard.

Tissue Pesticide Analysis

Pesticide concentrations in plasma were determined according to the method of Vukovich et al.[3] By this method, 1 ml of plasma is mixed with 20 μl of concentrated hydrochloric acid and 1.5 ml of n-hexane. The mixture is shaken for 20 minutes on a mechanical shaker and then centrifuged for 10 minutes to break up any emulsion formed. An aliquot is then injected into the gas chromatograph. Recoveries of radiolabeled samples of methyl parathion, parathion, and Thimet were 96 to 106%, 88 to 104%, and 82 to 102%, respectively. When radiolabeled methyl parathion samples were refrigerated for 3 days at 4°C, the recovery was 96 to 107%, indicating that refrigerated plasma could be stored for up to 3 days without loss or destruction of the compound.

To determine pesticide concentrations in frozen liver, about 4 g of tissue was placed in a Sorval Omni-mixer with an equal weight of anhydrous sodium sulfate and 20 ml of 10% isopropanol in hexane. The sample was blended for 5 minutes and then the extract was decanted through filter paper (Eaton-Dikeman, grade 512) into a Kuderna-Danish assembly that contained 6 g of anhydrous sodium sulfate. The extraction was repeated twice, and the pooled sodium sulfate was rinsed with 10 ml of 10% isopropanol in hexane. All the extracts were then concentrated down to a volume of approximately 3 ml over a steam bath.

Pesticide Purification

For parathion and methyl parathion, a column measuring 0.5" in diameter was packed to a depth of 10 cm with 60/100 mesh Florisil that had been maintained at 130°C for at least 24 hours. One inch of anhydrous sodium sulfate was added to the top of the column. The column was washed with 10 ml of hexane, leaving a minimal amount of hexane above the sodium sulfate at the top of the column. The pesticide concentrate was quantitatively transferred to the column using two 2.5-ml volumes of hexane. Elution of the column began using 15 ml of 10% diethyl ether in hexane, followed by 15 ml of 50% diethyl ether in hexane. The eluant was collected in a Kuderna-Danish assembly and concentrated in a steam bath to a few milliliters. Final evaporation to the desired volume was obtained using a gentle stream of nitrogen.

The purification of Thimet was essentially the same as for the parathions except that the Florisil column was packed to a depth of 5 cm, and elution was accomplished using 15 ml of 10% diethyl ether in hexane.

The purification of Guthion and Azodrin was somewhat different. A 0.5"-diameter column was packed with Florisil to a depth of 5 cm and topped off with anhydrous sodium sulfate. The column was washed with 20 ml of hexane, and then 9 ml of hexane was added to 1 ml of benzene extract of Guthion and put on the column. The pesticide was eluted using 100 ml of chloroform. The eluant was steam-evaporated to a small volume and then taken to dryness with a stream of nitrogen. The residue was dissolved in 1 ml of acetone for gas chromatography. The recoveries of Guthion and Azodrin averaged 94% and 65%, respectively.

Cholinesterase Assay

Cholinesterase activity was determined by the Hesterin method,[4] which is a modification of the procedure of Fleisher and Pope.[5] In the modified procedure, we used a 0.5-ml blood sample and a reagent blank in place of a water blank for the spectrophotometric determinations. The absorbance was read at 515 μm using a Beckman Model B spectrophotometer.

Animal Sacrifice

All animals in the fetotoxicity study were sacrificed on the twentieth day of gestation by asphyxiation in a chamber filled with CO_2. Each animal was examined for gross pathology, and the liver and gravid uterus were removed and weighed. The live fetuses then were removed and weighed, the uterus was examined, and the number of resorptions was noted. After the litters were weighed, each was divided into two equal groups of fetuses, one group fixed in Bouin's solution for necropsy and the other fixed in 70% ethanol for eventual clearing, staining, and skeletal analysis.

Pathology

Tissues taken for histopathological examination were immediately fixed in 10% neutral buffered formalin and later stained routinely with eosin and hematoxylin.

GENERAL TOXICOLOGY STUDIES - RESULTS

Exposure Chambers

Four exposure chambers, each of which can accommodate up to ten rats, were assembled in a series so that a single aerosol generator could provide exactly the same test material to each chamber. The aerosol concentrations in each chamber were altered by introducing metered dilutional air into the induction system before its entry into the inhalation chamber. The dilution ratio was governed by a metering orifice so that one of the ratios shown in Table 2 was theoretically possible at a constant pressure.

Table 2

DILUTION RATIOS IN THE EXPOSURE CHAMBERS

Orifice Diameter (in)	Dilution Factor	Chamber Dilutions			
		I	II	III	IV
0.081	$\sqrt{2}$	1	0.70	0.50	0.35
0.052	2	1	0.50	0.25	0.12
0.025	5	1	0.20	0.04	0.008

In practice, these ratios were achieved within a reasonable, expected concentration. Table 3 gives the concentrations achieved during some of the inhalation exposures to each of the five pesticides used in this study.

Table 3

CONCENTRATIONS OF VARIOUS PESTICIDES DISTRIBUTED TO FOUR INHALATION CHAMBERS SIMULTANEOUSLY BY A SPECIAL METERED DILUTIONAL DELIVERY SYSTEM

Pesticide	Dilution Ratio		Chamber Concentrations (mg/m^3)			
			I	II	III	IV
Parathion	$\sqrt{2}$	Actual	193	142	98	63
		Expected	214	151	107	75
Methyl parathion	$\sqrt{2}$	Actual	447	302	221	143
		Expected	500	353	250	176
Azodrin	$\sqrt{2}$	Actual	308	210	151	97
		Expected	300	211	149	104
	2	Actual	740	321	162	90
		Expected	710	355	178	89
Thimet	2	Actual	129	59	34	12
	2	Actual	114	53	20	10
	5	Actual	266	28	8	--
Guthion (20% in DMSO)	$\sqrt{2}$	Actual	250	155	106	70
		Expected	214	150	107	75
	2	Actual	86	24	13	7

Aerosol Particle Size

Aerosols were generated with an ultrasonic generator and passed through a cyclone separator and then into the distribution/dilution system, which was regulated by a 0.081-inch ratio orifice. The samples were taken from Chamber IV through the Royco particle counter.

All the aerosols were generated from neat technical-grade pesticides except Guthion. Guthion aerosols were generated from a 20% solution in DMSO. Table 4 shows the particle size distribution by count, the count median diameter, and the standard geometric deviation. The particle sizes generated were well within the respirable range of 0.3 to 3.0 μm, and the standard geometric deviation indicates that the distribution was not too heterodisperse for inhalation studies.

Purity of Pesticides

All pesticides used for these studies were provided by Battelle Memorial Institute, Columbus, Ohio. They were all technical-grade material. The purity of each compound was determined by gas chromatographic analysis after each inhalation exposure. Table 5 presents the results of these analyses. Parathion, Azodrin, and Guthion analyses were consistent and reproducible, whereas analyses of methyl parathion and Thimet were variable. No satisfactory explanation has been found for this apparent discrepancy.

Acute Toxicity Determinations

All the animals that received toxic or lethal doses of these organophosphate pesticides exhibited the common signs of cholinergic poisoning, regardless of the route of administration. That is, all exhibited salivation, lacrimation, exophthalmos, defecation, urination, and muscle fasciculations. The duration of the cholinergic signs was dose dependent with each compound but was not comparable among the several compounds tested. For example, a sublethal dose of parathion caused signs that lasted much longer than those produced by a comparable sublethal dose of Azodrin. Survivors of toxic doses of all pesticides had recovered completely 10 to 14 days after dosing.

Table 4

PARTICLE SIZE DISTRIBUTION OF AEROSOLS GENERATED FROM FIVE DIFFERENT PESTICIDES WITH AN ULTRASONIC NEBULIZER

	Percentage of Particle Size Distribution (μm)[2]						
Compound[1]	0.3-0.4	0.4-0.6	0.6-1.4	1.4-3.0	>3.0	CMD[3]	σg[4]
Parathion	16.0	27.6	43.0	13.3	0.1	0.68	1.8
Methyl parathion	13.4	25.2	50.0	11.4	0	0.71	1.7
Guthion	23.7	37.0	38.2	1.1	0	0.54	1.5
Thimet	23.0	34.0	35.6	7.4	0	0.57	1.8
Azodrin	17.7	28.8	35.2	18.1	0.1	0.69	1.95

[1] Particles were generated from neat solutions except Guthion, which was 20% in DMSO.

[2] Determined by the five stages of the Royco Particle Counter.

[3] Count median diameter.

[4] Standard geometric deviation.

Table 5

PURITY OF TECHNICAL GRADE PESTICIDES AS DETERMINED BY GAS CHROMATOGRAPHY DURING THE INHALATION EXPOSURE PERIODS

Compound	Range of Purity (%)
Parathion	88-94
Methyl parathion	70-80
Thimet	78-90
Azodrin	61-64
Guthion	72-73

Acute Oral Toxicity

Groups of ten male or female rats were treated with varying single doses of one pesticide by oral intubation. The animals were observed for the following 14 days for mortality and toxic signs. Table 6 presents the LD_{50} and 95% confidence limits calculated for each pesticide. Thimet was the most toxic of the five pesticides studied, with LD_{50}s of 3.7 and 1.4 mg/kg in male and female rats, respectively. Azodrin, with LD_{50}s of 35 and 20 mg/kg, respectively, for males and females, was the least toxic. Parathion, Thimet, and Azodrin were more toxic to females than to males, whereas Guthion and methyl parathion were about equitoxic to each sex.

Acute Intravenous Toxicity

Groups of ten male or female rats were treated with varying single doses of one of five pesticides by intravenous injection through the tail vein. The animals were observed for 14 days after treatment for toxic signs and mortality. Table 6 presents the LD_{50} and 95% confidence limits for each pesticide. Thimet was the most toxic of the pesticides by the intravenous route, having LD_{50}s of 2.2 and 1.2 mg/kg in male and female rats, respectively. Although the difference in LD_{50}s was very slight between the five pesticides, a small but significant difference between the LD_{50}s in males and females treated with Thimet and Azodrin was noted.

Acute Dermal Toxicity

Groups of ten male or female rats were treated with varying single doses of one of five pesticides by dermal application. The animals were observed during the treatment period for toxic signs and mortality and for 14 days thereafter. Table 6 presents the LD_{50} and 95% confidence limits for each pesticide. Thimet was the most toxic of the compounds when given by dermal application, having LD_{50}s of 9.3 and 3.9 mg/kg in male and female rats, respectively. Parathion was the next most toxic compound, with LD_{50}s of 49.4 and 19.5 mg/kg in males and females, respectively. Guthion was the least toxic of

Table 6

ACUTE TOXICITY OF PARATHION, METHYL PARATHION, THIMET, GUTHION, AND AZODRIN TO MALE AND FEMALE RATS WHEN GIVEN BY ORAL, INTRAVENOUS, DERMAL, AND INHALATION ROUTES

95% Confidence Limits in Parentheses

Compound	LD_{50} (mg/kg) or LC_{50} (mg/m^3)			
	Oral	Intravenous	Dermal	Inhalation
Parathion				
Male	14 (11-19)	6.4 (5.0-8.0)	49.4 (39.8-61.2)	1070 (754-1519)
Female	7.9 (6.3-9.8)	4.5 (3.1-6.6)	19.5 (17.0-22.4)	137 (125-151)
Methyl parathion				
Male	12 (8-16)	9.0 (6.6-11.3)	110 (91.8-131.8)	257 (188-352)
Female	18 (14-24)	14.5 (5.0-41.9)	120 (80-180)	287 (243-339)
Thimet				
Male	3.7 (2.6-5.3)	2.2 (1.9-2.6)	9.3 (7.9-11.0)	60 (52-69)
Female	1.4 (0.8-2.5)	1.2 (0.8-1.6)	3.9 (3.4-4.4)	11 (7-15)
Guthión				
Male	16 (13-19)	7.5 (5.1-11.1)	455 (301-687)	69 (62-77)
Female	18 (14-22)	7.5 (5.8-9.8)	222 (181-271)	79 (68-93)
Azodrin				
Male	35 (30-40)	11.9 (10.4-13.7)	210 (104-269)	162 (142-185)
Female	20 (17-23)	9.2 (8.7-9.7)	206 (104-407)	176 (159-195)

the pesticides in male rats, with an LD_{50} of 455 mg/kg. The LD_{50}s of Guthion in female rats and of Azodrin in male and female rats were nearly identical, ranging from 206 to 222 mg/kg. Differences between sexes in tolerance to the toxicity of Thimet, parathion, and Guthion were apparent, whereas no difference between sexes existed with Azodrin and methyl parathion.

Acute Inhalation Toxicity

Groups of ten male or female rats were exposed for 1 hour to atmospheres containing aerosols of one of five pesticides. The animals were observed for toxic signs and mortality during exposure and for 14 days thereafter. Chamber concentrations were verified during each exposure period for each pesticide by the appropriate analytical methodologies. Table 6 presents the acute LC_{50}s for a 1-hour exposure of the pesticides. Thimet was the most toxic of the pesticides when given by inhalation for 1 hour; the LC_{50}s were 60 and 11 mg/m^3 in male and female rats, respectively. Parathion was the least toxic in male rats, having an LC_{50} of 1070 mg/m^3. Methyl parathion was next, with LC_{50}s of 257 and 287 mg/m^3 in male and female rats. The LC_{50} of methyl parathion in female rats is in excellent agreement with the value found in another laboratory several years ago.[6] Females were much more susceptible than male rats to toxic effects of parathion and Thimet. No difference in tolerance between sexes was observed with methyl parathion, Guthion, or Azodrin.

Pesticide Levels in Tissues

We attempted to determine pesticide levels in blood plasma and liver at various times after various doses of pesticide administered by the intravenous, oral, dermal, or inhalation routes. Tables 7 and 8 summarize these data. The tables show that the amount of unchanged pesticide in plasma and liver is extremely variable under similar conditions of dose, time, and route of administration. We had anticipated that tissue levels of pesticide would correlate with mortality, but, in view of the consistently poor results obtained with these analyses, we abandoned this phase of the study.

Table 7

BLOOD PLASMA LEVELS OF PARATHION, METHYL PARATHION, OR THIMET IN RATS AT VARIOUS TIME INTERVALS AFTER ORAL, INTRAVENOUS, DERMAL, OR INHALATION ADMINISTRATION

Compound	Number of Rats	Route of Administration	Dose (mg/kg or mg/m^3)	Time After Dosing (min)	Pesticide Tissue Level ppb (Range)
Parathion	6	Intravenous	2.0	30	69 (15-166)
	5	Intravenous	5.1	60	208 (60-330)
	4	Oral	6.0	30	203 (14-287)
	7	Oral	10.0	30	< 4
	3	Oral	11.0	60	7.3 (3-20)
	5	Oral	13.5	60	6.8 (4-12)
	5	Oral	16.5	60	7.4 (3-11)
	5	Dermal	50	120	62 (38-90)
	5	Dermal	50	240	60 (30-79)
	3	Dermal	50	360	84 (76-102)
	4	Dermal	50	24 hrs	46 (35-56)
	6	Inhalation	346	60	24 (18-46)
Methyl parathion	3	Intravenous	8	5	4 (3-5)
	6	Intravenous	8	60	88 (60-120)
	9	Intravenous	10	20	3 (0.7-10.6)
	7	Oral	6	30	13 (<4-27)
	5	Oral	8	30	1.4 (0.9-2.2)
	6	Oral	8	120	41 (1.6-201)
	5	Oral	13	30	278 (61-518)
	12	Dermal	85	--	109 (59-246)
	6	Inhalation	152	155	29 (15-60)
Thimet	8	Intravenous	1.0	30	29 (6-114)
	7	Intravenous	1.6	30	16 (6-30)
	6	Intravenous	2.0	30	27 (7-49)
	7	Intravenous	2.5	30	32 (7-49)
	5	Oral	1.0	30	69 (23-92)
	6	Oral	2.7	60	13 (7-22)
	6	Oral	3.7	60	14 (5-33)
	6	Oral	5.0	60	23 (5-41)
	6	Inhalation	12	60	18 (3-38)

Table 8

TISSUE LEVELS OF PARATHION OR METHYL PARATHION
FOUND IN RAT LIVER AFTER ORAL OR INTRAVENOUS ADMINISTRATION

Compound	Number of Rats	Route of Administration	Dose (mg/kg)	Time After Dosing (min)	Pesticide Tissue Level ppb (Range)
Parathion	7	Intravenous	5.06	60	2.7 (<0.3-10.2)
	3	Oral	11.0	60	10.5 (6.5-16.2)
	5	Oral	13.5	60	21.9 (0.7-69.6)
	5	Oral	16.5	60	11.8 (<0.3-37.6)
Methyl parathion	5	Intravenous	8.0	5	8.9 (1.1-29.0)
	2	Intravenous	8.0	15	5.6 (2.9-8.2)
	7	Intravenous	8.0	60	0.7 (<0.2-1.3)
	7	Oral	8.0	30	11.9 (<0.3-46.7)
	6	Oral	8.0	120	16.2 (<0.3-51.8)
	6	Oral	9.0	60	12.9 (<0.3-34.4)
	6	Oral	12.0	60	8.5 (1.6-26.5)
	2	Oral	15.0	60	10.6 (7.6-13.5)

Blood Cholinesterase Activity

Blood was taken from groups of animals 1 hour after treatment with an approximately lethal dose of methyl parathion, Guthion, or Azodrin by various routes of administration. Table 9 summarizes the results of these studies. Blood cholinesterase inhibition was greatest after Azodrin treatment by all routes of administration. Slightly less inhibition was seen after methyl parathion treatment. Guthion was not as inhibitory as Azodrin or methyl parathion; the greatest inhibition obtained with Guthion was after inhalation exposure. The most surprising result was that little inhibition occurred with Guthion even after intravenous administration.

Pathology

No consistent pathological pattern was observed in animals given lethal doses of individual pesticides, although the rats that received methyl parathion and died within 1 hour of dosing exhibited hemorrhages in the thymus and lungs as well as dilation of the cerebral blood vessels. Because of the consistent absence of characteristic gross changes, only kidney, lung, brain, and skin of animals given the high-dose level by all routes of administration were examined microscopically. Of the 102 rats examined, no characteristic or recurrent histopathologic changes were found.

Lungs from animals that inhaled pesticide in the 1-hour LC_{50} exposures were fixed in 10% neutral buffered formalin, sectioned, and examined histologically. Seventy-five lungs were examined, and no specific recurrent changes were found. If hemorrhage, congestion, and edema are considered to represent pulmonary irritation, and if attention is focused on hemorrhage, Azodrin was the least irritating, since no hemorrhages were noted. Methyl parathion was intermediate, and the greatest irritation was observed with Thimet, Guthion, and parathion.

Table 9

CHOLINESTERASE INHIBITION IN THE WHOLE BLOOD OF MALE OR FEMALE RATS ONE HOUR AFTER TREATMENT WITH METHYL PARATHION, GUTHION, OR AZODRIN

Compound	Route of Administration	Sex	Dose (mg/kg)	Average Percentage of Cholinesterase Inhibition (range in parentheses)
Methyl parathion	Oral	Male	11.7	59 (52-73)
	Intravenous	Male	6.6	76 (67-81)
	Dermal	Male	110	84 (78-93)
	Dermal	Male	85	64 (50-73)[1]
	Dermal	Female	85	57 (49-67)[1]
	Inhalation	Male	264	59 (53-61)
Guthion	Oral	Female	13	8 (7-20)
	Intravenous	Male	5.6	26 (9-38)
	Intravenous	Female	5.6	14 (7-19)
	Inhalation	Male	39	41 (27-59)
Azodrin	Oral	Male	25	82 (63-89)
	Oral	Female	15	89 (83-94)
	Intravenous	Male	12	92 (91-93)
	Intravenous	Female	9	79 (65-90)
	Inhalation	Male	156	74 (73-77)
	Inhalation	Female	192	69 (62-68)

[1] Blood taken six hours after treatment.

DISCUSSION

Aerosol Generation and Characterization

The generation of a well-defined, compatible, and reproducible aerosol is a critical requirement for attaining reliable and meaningful inhalation toxicity data. Such generation was achieved in these studies by use of the ultrasonic generator. The aerosol generated was passed into a cyclone separator, and only the desired particle sizes were routed into the inhalation chambers. This was confirmed by analysis of the aerosols of each of the five pesticides with the Royco particle counter. All the aerosols had a count median diameter of 0.5 to 0.65 μm. The respirable range is considered to be 0.3 to 3.0 μm.[7] In addition, the standard geometric deviation (σg) of less than 2.0 indicates that, although the aerosols were heterodisperse, nearly all the particles were well within the respirable size range.

Exposure Chambers

A unique exposure chamber system was constructed for these studies. It allowed simultaneous exposure of up to four groups of animals to an aerosol from a single generator. This was made possible by serial dilution of the generated aerosol just before its entry into each chamber. The dilution system was regulated by a fixed orifice, which gave a constant dilutional ratio between each chamber when operated at a constant flow pressure. By careful selection of ratioing orifices, we could obtain an LC_{50} for a compound in one sex of one species of animals from a single 1-hour exposure. However, a much more useful application of this exposure system is in studies requiring repeated exposures at multiple dose levels. The researcher can easily duplicate these exposures from day to day by merely setting the flow rates and pressures to predetermined positions each day.

Moreover, these exposure chambers provide nose-only exposure to the aerosol. This factor is important if the compound being studied can easily be absorbed through the skin. Nose-only exposure is also important if the mechanism of action or the metabolism of a compound is different when administered by different routes.

Acute Toxicity Studies

Thimet was the most toxic of the five pesticides studied, regardless of the route of administration. It was also two to three times less toxic to males than to females by all routes except inhalation, by which route it was four times less toxic. Parathion was less toxic to males than to females when administered orally, dermally, or by inhalation but not by intravenous injection. Guthion was less toxic to males only after dermal application, and Azodrin was less toxic to males only by oral or intravenous administration. No difference in tolerance between sexes was noted with methyl parathion.

Comparison of the intravenous, oral, and dermal toxicity of each compound reveals that, on a mg/kg basis, the intravenous route was most toxic, followed by the oral and then the dermal routes. It is difficult to compare the LC_{50} of the inhalation exposures with the LD_{50} of the intravenous dosing studies. However, certain assumptions can be made that permit a conversion of the LC_{50} data from a mg/m^3 to a mg/kg basis. For example, we know that a 200-g rat has a respiratory minute volume of about 80 ml/min. Therefore, a 200-g rat will breathe about 4,800 ml in 60 minutes, or a total of 4.8 liters of the test atmosphere. A reasonable assumption is that about 20% of an aerosol with a particle size range of 0.3 to 3.0 μm will be deposited in the bronchial or alveolar area of the lung.[7] The following relationship is then suggested:

LC_{50} x respired fraction of LC_{50} x body weight (kg) x percentage of deposition = LD_{50} , or

LC_{50} (mg/m^3) x 4.8 liters/1000 liters x 200 g/1000 g x 0.20 = LD_{50} in mg/kg .

Calculation of the LD_{50} for each pesticide based on the above relationship would indicate that, with the exception of parathion in male rats, the inhalation route of pesticide administration was 7 to 24 times more toxic than intravenous dosing, as suggested by the data presented in Table 10.

There are two possible reasons for the greater inhalation toxicity. First, material entering the body via the lung enters the left heart and then is distributed throughout the tissues before it reaches the liver, where the major detoxifying systems are located. Therefore, primary target organs and/or tissues might receive a relatively greater portion of a given dose of pesticide by inhalation than by the oral or intravenous routes, by which the dose would reach the liver first. Second, the toxicity of organothiophosphates is greatly increased when the organothiophosphates are converted to their oxygen analogs. Neal et al.[7,8] have shown that rabbit lung, *in vitro*, can convert parathion to paraoxon, its oxygen analog; rabbit lung also can slowly detoxify paraoxon to *p*-nitrophenol, although the lung contains only about 3% of the enzyme activity found in the liver.

This entire metabolic process can be altered (induced) by a number of compounds including DDT, barbiturates, and steroid hormones (particularly testosterone), which probably accounts for the differences in tolerance between sexes seen with parathion and Thimet.

Tissue Distribution

The attempt to recover unchanged parathion, methyl parathion, and Thimet from plasma or liver after dosing by various routes of administration was unsuccessful. Two possible reasons for this are: (1) Because the compounds tested are rapidly converted to their oxygen analogs by plasma and particularly liver enzymes, they would not be detected; and (2) these compounds are firmly bound not only to acetylcholinesterases but also to other nonspecific esterases. They probably could not be removed from their bound esterases without taking measures that would cause further hydrolysis of the pesticide molecule. Perhaps the only way to measure plasma half-life or liver

Table 10

A COMPARISON OF THE ACUTE INTRAVENOUS LD_{50} AND THE ACUTE INHALATION LC_{50} OF PARATHION, METHYL PARATHION, THIMET, GUTHION, AND AZODRIN IN MALE AND FEMALE RATS

Compound	Sex	Intravenous LD_{50} (mg/kg)	Calculated Inhalation LD_{50} (mg/kg)	Intravenous LD_{50}/ Inhalation LD_{50}
Parathion	Male	6.4	5.14	1.2
	Female	4.5	0.66	6.8
Methyl parathion	Male	9.0	1.23	7.3
	Female	14.5	1.38	10.5
Thimet	Male	2.2	0.29	7.6
	Female	1.2	0.05	24
Guthion	Male	7.5	0.33	23
	Female	7.5	0.38	20
Azodrin	Male	11.9	0.78	15
	Female	9.2	0.85	10.8

content with these compounds is to use radioactive material such as a double label with ^{35}S and ^{32}P isotopes or an uniformly labeled ^{14}C compound.

Cholinesterase Inhibition

Clearly death of animals treated with methyl parathion, Guthion, or Azodrin was not correlated to the degree of cholinesterase inhibition in the blood. Although a moderate to great cholinesterase inhibition occurred in rats treated with lethal doses of parathion and Azodrin, very little inhibition was seen in the Guthion-treated animals except by inhalation. However, this may reflect the fact that the compounds have different mechanisms of producing mortality as well as a similar action of cholinesterase inhibition. Because whole blood contains both true and pseudo cholinesterases, a better correlation might have been found if one or the other had been measured alone. Another possibility is that the pesticides might all produce a significant cholinesterase inhibition in a critical location, the central nervous system for example, to produce mortality by a common mode of action.

Pathology

No significant gross or histopathological lesions attributable to pesticide treatment were found in any of the animals examined. These observations, therefore, support the position that the primary hazard from treatment with these five pesticides is a biochemical lesion.

SUMMARY

A method was developed for generating pesticide aerosols in the respirable particle size range of 0.3 to 3.0 μm. Microanalytical techniques also were developed for determining pesticide concentrations of parathion, methyl parathion, Thimet, Guthion, and Azodrin by gas chromatography. Methods were developed for extracting these five pesticides from rat blood and liver.

A unique inhalation exposure chamber system was constructed that permits the simultaneous exposure of up to four groups of animals to four different predetermined concentrations of an aerosol from a single generator source.

In male and female rats, the acute oral, dermal, and intravenous LD_{50} and the acute inhalation LC_{50} were determined and compared for parathion, methyl parathion, Thimet, Guthion, and Azodrin. The inhalation route of administration of all five pesticides appeared to be the most toxic, followed by the intravenous and oral routes. Thimet was the most toxic of the five compounds by all routes of administration. Females were more susceptible to Thimet and parathion than males. There was no correlation between the tissue levels of pesticide and the dose or the route of administration, nor was there correlation between the inhibition of cholinesterase activity in blood and mortality.

No significant pathological lesions that could be attributed to treatment developed in the animals treated with any of the five pesticides.

FETOTOXICITY STUDIES - RESULTS AND DISCUSSION

Chloroform

All the animals exposed to 20.1 ± 1.2 mg/l (the highest level) fell asleep within a few minutes after initiation of exposure and continued to sleep throughout the exposure period. Some of the animals exposed to 10.9 ± 1.0 mg/l (the mod-dose) of chloroform occasionally appeared to be asleep. All animals in the low-level exposure chamber and the control chamber responded readily to light tapping on the chamber wall or to a bright light stimulus. One animal receiving the highest level of chloroform died during the night of the twelfth day of gestation. At autopsy, autolysis precluded any determination of resorptions at the nine implantation sites.

During the exposure period, the average body weights of the animals receiving the high dose of chloroform decreased, whereas the weights of those receiving the low dose did not change. During the same period, the control rats gained an average of 43 grams. Table 11 summarizes the body weight data.

Food consumption was reduced in all the treated groups during the chloroform exposure, and this response seemed to be dose-related. Food consumption returned to normal on the day chloroform exposures were completed. These data are summarized in Table 12.

At autopsy, no gross pathologic lesions were noted that could be attributed to the treatment. Table 13 lists the litter sizes, number of live and dead/resorbed fetuses, fetal weights, and the total number of pregnancies per treatment group. The number of resorptions increased in the highest treatment group and in the restricted-food controls. The average fetal weights were somewhat less in the treated groups compared with the air controls. The overall fetal development of the restricted-food controls were significantly less than those of the air controls. No treatment-related changes in fetal ossification or occurrence of supernumerary ribs were noted. No gross defects were seen in any dose group.

Table 11

AVERAGE BODY WEIGHTS OF PREGNANT RATS EXPOSED TO CHLOROFORM ATMOSPHERES FOR 1 HOUR DAILY DURING DAYS 7 THROUGH 14 OF GESTATION

	Average Body Weights† During Days of Gestation			
Treatment Group*	1	6	15	20
20.1 ± 1.2 mg/l	202 ± 8	221 ± 7	192 ± 25‡	252 ± 41‡
10.9 ± 1.7 mg/l	208 ± 6	233 ± 9	213 ± 16	280 ± 25
4.6 ± 1.0 mg/l	203 ± 7	223 ± 6	224 ± 12	280 ± 32
Air controls	209 ± 7	231 ± 8	275 ± 12	335 ± 13
Restricted food controls	208 ± 5	230 ± 6	195 ± 13	269 ± 32

* Ten animals per treatment group.
† In grams ± the standard error.
‡ Nine animals per group.

Table 12

AVERAGE DAILY FOOD CONSUMPTION OF PREGNANT RATS EXPOSED TO CHLOROFORM ATMOSPHERES FOR 1 HOUR DAILY DURING DAYS 7 THROUGH 14 OF GESTATION

	Average Daily Food Consumption (g/rat) During Days of Gestation		
Treatment Group*	1-7	8-14	15-20
20.1 ± 1.2 mg/l	16.0	5.8†	21.0†
10.9 ± 1.7 mg/l	16.6	9.2	21.0
4.6 ± 1.0 mg/l	15.4	11.6	24.6
Air controls	16.4	19.8	24.2
Restricted food controls	16.0	5.8	21.0

* Ten animals per group.
† Nine animals per group.

Table 13

LITTER SIZE, RESORPTIONS, LIVE FETUSES, AND FETAL WEIGHTS AT DAY 20 OF GESTATION IN PREGNANT RATS EXPOSED TO CHLOROFORM ATMOSPHERES FOR 1 HOUR DAILY DURING DAYS 7 THROUGH 14 OF GESTATION

	Control	4.6 ± 1.0 mg/m^3	10.9 ± 1.7 mg/m^3	20.1 ± 1.2 mg/m^3	RFC[b]
No. pregnant (term)	10	9	10	8	10
Av. implants	10.6 ± 0.3	9.9 ± 0.8	10.6 ± 0.5	10.0 ± 0.8	12.5 ± 0.6
Av. mortality (%)	10 ± 8	3 ± 2	16 ± 6	45 ± 21[a]	32 ± 14[a]
Av. weight (g)	4.0 ± 0.1	3.6 ± 0.2	3.9 ± 0.1	3.7 ± 0.1[a]	3.0 ± 0.1[a]
Av. no. sternal ossification centers	6.0 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	5.5 ± 0.2
Av. no. caudal ossification centers	4.7 ± 0.1	4.3 ± 0.4	5.0 ± 0.2	4.5 ± 0.2	3.3 ± 0.2[a]
Supernumerary ribs (%)	19 ± 10	10 ± 6	14 ± 8	17 ± 12	5 ± 3

[a] $p<0.05$

[b] Restricted Food Controls

Ethylene Thiourea

None of the animals exposed to ethylene thiourea exhibited any toxic signs during the daily inhalation exposure periods. However, as shown in Table 14, those animals exposed to 120.4 mg/m^3 (the highest dose) appeared to have a slight decrease in weight gain, as compared with the other dosage groups. Statistically, they were not different from the controls. Food consumption also was less in the highest group, as shown in Table 15. However, one animal was not pregnant, and another had the total litter resorbed, and this may account for the observed difference.

At autopsy, no lesions were observed that could be attributed to the treatment.

Table 16 lists the litter sizes, number of live and dead fetuses, fetal weights, and the total number of pregnancies per treatment group. The number of resorptions was highest in the group receiving 120 mg/m^3 (the highest treatment group) of ethylene thiourea. The number of resorptions decreased as the dose decreased and was least in the air controls. There was a significant ($p<0.01$) dose-related reduction in fetal weight and degree of ossification in the treated groups. The fetal weights of the restricted food controls was significantly less than the air controls--3.4 ± 0.2 g versus 4.1 ± 0.2 g. No terata were observed in any of the treatment groups.

Thimet

The animals exposed to 1.94 mg/m^3 (the highest concentration) of Thimet exhibited toxic signs and mortality during the eight daily exposures. All the animals exhibited tremors, lacrimation, and exophthalmos. A total of five animals died--one after the third, fourth, sixth, seventh, and eighth exposures, respectively. Two rats that died had bloody material in their intestines and bladder. All the animals that died were pregnant, but the one that died after the eighth exposure appeared to be resorbing her entire litter.

Table 14

AVERAGE BODY WEIGHTS OF PREGNANT RATS EXPOSED TO ETHYLENE THIOUREA AEROSOLS FOR 3 HOURS DAILY DURING DAYS 7 THROUGH 14 OF GESTATION

Treatment Group*	Average Body Weights† During Days of Gestation			
	1	6	15	20
120.4 ± 8.0 mg/m^3‡	224 ± 6	246 ± 8	278 ± 9	336 ± 17
55.5 ± 5.5 mg/m^3	226 ± 8	250 ± 7	289 ± 18	349 ± 17
27.2 ± 3.1 mg/m^3	225 ± 8	248 ± 8	285 ± 8	347 ± 17
Air controls	224 ± 7	248 ± 9	283 ± 15	349 ± 23
Restricted food controls	218 ± 6	240 ± 12	262 ± 14§	323 ± 17§

* Ten animals per group unless otherwise indicated.
† In grams ± the standard error.
‡ Eight animals per group.
§ Nine animals per group.

Table 15

AVERAGE DAILY FOOD CONSUMPTION OF PREGNANT RATS EXPOSED TO ETHYLENE THIOUREA AEROSOLS FOR 3 HOURS DAILY DURING DAYS 7 THROUGH 14 OF GESTATION

Treatment Group*	Average Daily Food Consumption (g/rat) During Days of Gestation		
	1 to 7	8 to 14	15 to 20
120.4 ± 8.0 mg/m^3	16.8	17.5	20.9
55.5 ± 5.5 mg/m^3	18.8	20.8	24.4
27.2 ± 3.1 mg/m^3	17.7	19.2	25.2
Air controls	18.3	19.3	24.3
Restricted food controls	16.8	17.5	20.9

* Ten animals per group.

Table 16

LITTER SIZE, RESORPTION,S LIVE FETUSES, AND FETAL WEIGHTS
AT DAY 20 OF GESTATION IN PREGNANT RATS EXPOSED TO ETHYLENE THIOUREA AEROSOLS
FOR 3 HOURS DAILY DURING DAYS 7 THROUGH 14 OF GESTATION

	Control	27.2 ± 3.1 mg/m^3	55.5 ± 5.5 mg/m^3	120.4 ± 8.0 mg/m^3	RFC[d]
No. term pregnant	10	10	10	9	9
Av. no. implants	11.5 ± 0.3	11.1 ± 0.5	11.5 ± 0.4	10.8 ± 0.6	12.3 ± 0.4
Av. mortality (%)	2.8 ± 2.0	4.5 ± 2.3	7.6 ± 3.2	19.1 ± 10.5[a]	6.1 ± 2.8
Av. weight (g)	4.1 ± 0.1	4.2 ± 0.1	4.0 ± 0.1	3.7 ± 0.1[b]	3.4 ± 0.1[b]
Av. no. sternal ossification centers	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.0	5.8 ± 0.1	-
Av. no. caudal ossification centers	5.4 ± 0.2	4.8 ± 0.2[a]	5.0 ± 0.1	3.9 ± 0.1[c]	-
Av. supernumerary ribs (%)	20 ± 8	13 ± 4	29 ± 11	16 ± 5	-

[a] $p<0.05$
[b] $p<0.01$
[c] $p<0.001$
[d] Restricted Food Controls

Table 17 presents the average body weights of pregnant rats exposed to Thimet aerosols. No differences in weight gain were noted that could not be accounted for on the basis of the number of pregnancies in each group, except for an unexplained weight loss in the restricted-food controls on the fifteenth day of gestation.

Food consumption, presented in Table 18, was not different among any of the groups, considering the number of pregnancies in each group.

Table 19 lists the litter size, number of live and dead/resorbed fetuses, fetal weights, and number of pregnancies in each group. The highest dose of Thimet produced the highest fetal mortality as well as maternal mortality. Also, the average fetal weight at the highest dose seemed to be slightly greater than the other groups. No other fetal effects were seen. These observations were not the result of restricted food intake or solvent (xylene) toxicity.

Bromacil

No toxic signs were noted in any of the animals exposed to Bromacil or to the DMSO solvent during the 8 days of treatment. Table 20 presents the body weights of all the animals in this study. There is no difference in the weight gain of any of the groups that cannot be explained on the basis of nonpregnant animals in the group.

The food consumption, presented in Table 21, reflects the weight gains, and there is no difference among any of the groups. No restricted-food controls were included in the Bromacil study.

Table 22 lists the litter size, number of live and dead/resorbed fetuses, average fetal weight, and the number of pregnancies in each group. The group receiving 165 mg/m^3 (highest dose) of Bromacil appeared to have a slightly higher percentage of resorptions, although the air controls and DMSO controls have a slightly higher resorption rate than some of our previous control groups. Significant ($p<0.01$) dose-related reductions in fetal weight and caudal ossification were seen in treated groups. No gross pathology was noted in any of the groups.

Table 17

AVERAGE BODY WEIGHTS OF PREGNANT RATS EXPOSED TO THIMET AEROSOLS FOR 1 HOUR DAILY DURING DAYS 7 THROUGH 14 OF GESTATION

Treatment Group*	Average Body Weights† During Days of Gestation			
	1	6	15	20
1.94 ± 0.48 mg/m^3	214 ± 5	240 ± 5	278 ± 12‡	311 ± 19‡
0.40 ± 0.15 mg/m^3	216 ± 5	237 ± 6	268 ± 7	337 ± 13
0.15 ± 0.04 mg/m^3	214 ± 5	235 ± 6	271 ± 7	333 ± 12
Restricted food controls	227 ± 2	245 ± 3	236 ± 6	302 ± 10
Air controls	210 ± 5	231 ± 5	257 ± 11	311 ± 18
Xylene controls	---	219 ± 3	254 ± 6	300 ± 16

* Ten animals per group.
† In grams ± the standard error.
‡ Five animals died during the inhalation exposure.

Table 18

AVERAGE DAILY FOOD CONSUMPTION OF PREGNANT RATS EXPOSED TO THIMET AEROSOLS FOR 1 HOUR DAILY DURING DAYS 7 THROUGH 14 OF GESTATION

Treatment Group*	Average Daily Food Consumption (g/rat) During Days of Gestation		
	1-7	8-14	15-20
1.94 ± 0.48 mg/m^3	19.0	22†	26.4†
0.40 ± 0.15 mg/m^3	19.2	20.1	24.4
0.15 ± 0.04 mg/m^3	18.2	19.7	23.1
Restricted food controls	17.3	17.5	--
Air controls	17.3	18.1	20.8
Xylene controls	18.4	19.2	22.1

* Ten animals per group unless otherwise indicated.
† Five animals per group.

Table 19

LITTER SIZE, RESORPTIONS, LIVE FETUSES, AND FETAL WEIGHTS AT DAY 20 OF GESTATION IN PREGNANT RATS EXPOSED TO THIMET AEROSOLS FOR 1 HOUR DAILY DURING DAYS 7 THROUGH 14 OF GESTATION

	Control	0.15 ± 0.04 mg/m^3	0.40 ± 0.15 mg/m^3	1.94 ± 0.48 mg/m^3	RFC[d]	Xylene
No. pregnant (term)	15	10	9	5[e]	8	7
Av. implants	10.1 ± 0.7	10.0 ± 1.1	11.7 ± 0.2	10.2 ± 2.1	9.5 ± 1.1	11.6 ± 1.1
Av. mortality (%)	3.8 ± 1.6	5.7 ± 2.2	3.6 ± 1.9	30.8 ± 18.7	6.6 ± 2.5	7.4 ± 3.2
Av. weight (g)	3.8 ± 0.1	3.9 ± 0.1	3.8 ± 0.1	4.1 ± 0.2[a]	3.7 ± 0.1	3.9 ± 0.1
Av. no. sternal ossification centers	6.0 ± 0.0	5.9 ± 0.1	5.9 ± 0.1	5.8 ± 0.2	6.0 ± 0.0	6.0 ± 0.0
Av. no. caudal ossification centers	4.4 ± 0.1	4.5 ± 0.2	4.3 ± 0.2	4.3 ± 0.4	4.4 ± 0.2	4.4 ± 0.2
Supernumerary ribs (%)	9.9 ± 5.7	12.2 ± 5.2	5.9 ± 3.0	3.4 ± 7.6	13.5 ± 9.6	0

[a]p 0.05

[b]p 0.01

[c]p 0.001

[d]Restricted Food Controls

[e]Five additional rats died during the exposures to Thimet. All rats were pregnant, but they were not evaluated for live or dead fetuses.

Table 20

AVERAGE BODY WEIGHTS OF PREGNANT RATS EXPOSED TO BROMACIL AEROSOLS FOR 2 HOURS DAILY DURING DAYS 7 THROUGH 14 OF GESTATION

Treatment Group*	Average Body Weights† During Days of Gestation			
	1	6	15	20
165 ± 6 mg/m^3	195 ± 16	219 ± 17	251 ± 24	307 ± 41
78 ± 3 mg/m^3	206 ± 15	227 ± 13	253 ± 19	305 ± 37
38 ± 2 mg/m^3	200 ± 11	222 ± 10	258 ± 14	316 ± 19
DMSO controls	202 ± 13	227 ± 13	262 ± 15	318 ± 30
Air controls	198 ± 7	227 ± 11	265 ± 13	326 ± 23

* Ten animals per group.
† In grams ± the standard error.

Table 21

AVERAGE DAILY FOOD CONSUMPTION OF PREGNANT RATS EXPOSED TO BROMACIL AEROSOLS FOR 2 HOURS DAILY DURING DAYS 7 THROUGH 14 OF GESTATION

Treatment Group*	Average Daily Food Consumption (g/rat) During Days of Gestation		
	1-7	8-14	15-20
165 ± 6 mg/m^3	16.9	19.7	22.9
78 ± 3 mg/m^3	17.8	19.5	22.5
38 ± 2 mg/m^3	17.3	20.0	22.9
DMSO controls	18.8	15.5	24.2
Air controls	19.2	16.4	24.0

* Ten animals per group.

Table 22

LITTER SIZE, RESORPTIONS, LIVE FETUSES, AND FETAL WEIGHTS AT DAY 20 OF GESTATION IN PREGNANT RATS EXPOSED TO BROMACIL AEROSOLS FOR 2 HOURS DAILY DURING DAYS 7 THROUGH 14 OF GESTATION

	Control	38 ± 2 mg/m^3	78 ± 3 mg/m^3	165 ± 6 mg/m^3	DMSO Control	Air Control
No. pregnant (term)	19	10	8	9	9	10
Av. implants	10.8 ± 0.4	11.2 ± 0.5	10.8 ± 0.8	10.9 ± 0.7	10.9	10.7
Av. mortality (%)	8.9 ± 3.1	4.7 ± 3.0	5.3 ± 2.2	7.6 ± 3.7	9.2	9.4
Av. weight (g)	4.3 ± 0.04	4.1 ± 0.09[a]	3.9 ± 0.07[b]	4.0 ± 0.09[a]	4.3 ± 0.2	4.3 ± 0.3
Av. no. sternal ossification centers	6.0 ± 0.02	6.0 ± 0.0	5.9 ± 0.03	6.0 ± 0.03	-	-
Av. no. caudal ossification centers	5.2 ± 0.2	4.5 ± 0.2[b]	4.8 ± 0.1[a]	4.7 ± 0.2[a]	-	-
Supernumerary ribs (%)	20.2 ± 4.4	2.0 ± 2.0	13.3 ± 6.5	20.0 ± 8.8	-	-

[a] $p<0.05$
[b] $p<0.01$

Simazine

Animals exposed to the highest dose of Simazine usually did not appear as active after each inhalation exposure as those in the other groups. However, they all appeared normal on the day after exposure when they were being prepared for the next inhalation treatment. The average body weights, shown in Table 23, and the food consumption of the animals, shown in Table 24, do not indicate any differences among the treated and the control groups. No gross pathology was noted at the time of sacrifice.

Table 25 lists the litter size, number of live and dead/resorbed fetuses, average fetal weight, and number of pregnancies in each group. There were no differences in the number of resorptions among the groups. The fetal weight and degree of caudal ossification were less in the treated groups compared to the controls. No treatment-relative terata were noted in any group.

SRI's chloroform study was initiated to reproduce the results of Schwetz et al.[1] Those investigators exposed animals to 30, 100, and 300 ppm chloroform for 7 hr/day, whereas our exposures were much higher--950, 220, and 4100 ppm

$$\left(\frac{mg/m^3}{1000} \quad \left\{\frac{24,450}{mol\ wt}\right\} = \quad ppm\right) \quad .$$

However, our exposure time was reduced to 1 hour daily to reduce the stress on the animals. The results obtained with our highest dose of 4100 ppm for 1 hr/day are similar to those obtained by Schwetz et al.[1] at 300 ppm for 7 hr/day. The percentage of resorptions observed by Schwetz et al. was 61%, and ours was 44%. Their average live litter size was 4.7, whereas ours was 5.6. Thus, we essentially have repeated their experimental results using different exposure times and chloroform concentrations.

Table 23

AVERAGE BODY WEIGHTS OF PREGNANT RATS EXPOSED TO SIMAZINE AEROSOLS FOR 2 HOURS DAILY DURING DAYS 7 THROUGH 14 OF GESTATION

Treatment Group*	Average Body Weights† During Days of Gestation			
	1	6	15	20
317 ± 89 mg/m^3	182 ± 7	211 ± 8	238 ± 13	309 ± 31
77 ± 56 mg/m^3	183 ± 9	212 ± 9	239 ± 11	316 ± 22
17 ± 12 mg/m^3	186 ± 7	217 ± 6	241 ± 14	316 ± 22
DMSO controls	202 ± 13	227 ± 13	262 ± 15	318 ± 30
Air controls	198 ± 7	227 ± 11	265 ± 13	326 ± 23

* Ten animals per group.
† In grams ± the standard error.

Table 24

AVERAGE DAILY FOOD CONSUMPTION OF PREGNANT RATS EXPOSED TO SIMAZINE AEROSOLS FOR 2 HOURS DAILY DURING DAYS 7 THROUGH 14 OF GESTATION

Treatment Group*	Average Daily Food Consumption (g/rat) During Days of Gestation		
	1-7	8-14	15-20
317 ± 89 mg/m^3	12.4	19.2	24.5
77 ± 56 mg/m^3	12.5	19.1	24.8
17 ± 12 mg/m^3	12.5	19.2	23.9
DMSO controls	18.8	15.5	24.2
Air controls	19.2	16.4	24.0

* Ten animals per group.

Table 25

LITTER SIZE, RESORPTIONS, LIVE FETUSES, AND FETAL WEIGHTS AT DAY 20 OF GESTATION IN PREGNANT RATS EXPOSED TO SIMAZINE AEROSOLS FOR 2 HOURS DAILY DURING DAYS 7 THROUGH 14 OF GESTATION

	Control	17 ± 12 mg/m^3	77 ± 56 mg/m^3	317 ± 89 mg/m^3	DMSO Control	Air Control
No. pregnant (term)	19	10	10	9	9	10
Av. implants	10.8 ± 0.4	10.6 ± 1.0	10.8 ± 0.7	11.3 ± 0.5	10.9	10.7
Av. mortality (%)	8.9 ± 3.1	15.3 ± 9.7	8.7 ± 3.0	8.3 ± 3.4	9.2	9.4
Av. weight (g)	4.3 ± 0.04	3.8 ± 0.06[b]	4.0 ± 0.09[a]	3.7 ± 0.06[b]	4.3 ± 0.2	4.3 ± 0.3
Av. no. sternal ossification centers	6.0 ± 0.02	5.9 ± 0.1	6.0 ± 0.01	5.9 ± 0.03	-	-
Av. no. caudal ossification centers	5.2 ± 0.2	4.5 ± 0.2[a]	4.8 ± 0.2	4.4 ± 0.2[b]	-	-
Supernumerary ribs (%)	20.2 ± 4.4	4.1 ± 2.7	11.7 ± 6.6	25.6 ± 8.8	-	-

[a] $p<0.01$

[b] $p<0.001$

Ethylene thiourea exposures were established to reproduce the results of Khera,[2] who orally dosed rats and rabbits with 5, 10, 20, 40, and 80 mg/kg, respectively. To compare Khera's data with those from our inhalation studies, we converted our data to an mg/kg basis. Assuming that a 200-g rat has a respiratory volume of 80 ml/minute,[11] then in 60 minutes the rat will have breathed 4800 ml, or 4.8 liters, of air. If we assume 100% absorption/deposition of material in the respiratory tract, we can then make the following estimate of the daily dose:

$$\text{Aerosol concentration } (\text{mg/m}^3)\ \frac{80\ \text{ml/min} \times \text{exposure time (min)}}{1000}$$

$$\times\ \frac{1000\ \text{g}}{\text{rat wt. (g)}} = \text{daily dose in mg/kg.}$$

Table 26 shows the conversion of all the aerosol concentrations to mg/kg. On this basis, our highest possible dose of ethylene thiourea was 8.67 mg/kg. Khera found only a decrease in mean fetal weight in the 80-mg/kg treatment group, whereas we found a slight decrease in fetal weight plus a slight increase in fetal mortality in our highest dose group (120.4 mg/m^3, or 8.67 mg/kg). This is not surprising, since inhalation administration usually is much more efficient, comparing closely to intravenous administration.

Thimet was the most toxic of the compounds tested for teratogenicity. The major prenatal observations were a slight increase in fetal mortality in the high-dose group and possibly a slight increase in fetal weight. Maternal mortality precluded exposure to higher doses.

Bromacil or Simazine did not produce any prenatal changes of weight or fetal mortality in any of the treated groups. This was not unexpected, since both these compounds are relatively insoluble.

Table 26

CONVERSION OF DAILY MEAN AEROSOL CONCENTRATIONS TO DOSAGES IN mg/kg*

Compound	Daily Exposure Time (hr)	Aerosol Concentration	Daily Dose (mg/kg)
Chloroform	1	20.1 mg/l (4100 ppm)	480
	1	10.9 mg/l (2200 ppm)	260
	1	4.6 mg/l (950 ppm)	110
Ethylene Thiourea	3	120.4 mg/m^3	8.67
	3	55.5 mg/m^3	4.00
	3	27.2 mg/m^3	1.96
Thimet	1	1.94 mg/m^3	0.047
	1	0.40 mg/m^3	0.047
	1	0.15 mg/m^3	0.004
Bromacil	2	165 mg/m^3	7.92
	2	78 mg/m^3	3.75
	2	38 mg/m^3	1.83
Simazine	2	317 mg/m^3	15.22
	2	77 mg/m^3	3.70
	2	17 mg/m^3	0.82

* Assuming a respiratory volume of 80 ml/min for a 200-g rat and 100% deposition and absorption of the aerosol.

SUMMARY

Pregnant rats were exposed daily to five different chemicals by the inhalation route from day 7 through day 14 of gestation.

Chloroform, ethylene thiourea, and Thimet caused some prenatal changes of increased fetal mortality and possibly some fetal weight loss.

Bromacil and Simazine had no prenatal effect on rats under these experimental conditions.

No compounds produced treatment-related teratogenic effects.

None of the dams exhibited gross pathological changes that could be attributed to the inhalation exposures.

RECOMMENDATIONS

These studies suggest that inhalation is perhaps the most sensitive route of administering pesticides. Furthermore, other routes of administration do not necessarily predict the toxicity of a chemical administered by inhalation. Since inhalation is probably the most common route of exposure, inhalation toxicity studies should be performed on all pesticides that are volatile or prepared/administered as a dust or aerosol.

Although no teratology was observed in these studies, further work should continue in this area because inhalation is the route of exposure most often encountered.

These studies should be extended to include inhalation mutagenesis with compounds that are suspected of producing mutations. Compounds tested by oral or percutaneous administration may be poorly absorbed or destroyed (in the gut). Inhalation more nearly represents an intravenous dose, and many materials are readily transported across the alveolar membranes into the circulation.

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16. ABSTRACT

A method was developed for generating pesticide aerosols within the respirable particle size range of 0.3 to 3.0μm. Analytical methods were established for determining pesticide concentrations in chamber air samples and in tissues. A unique chamber exposure system was developed that permitted the simultaneous exposure of four different groups of rats to four different concentrations of pesticide from a single generation source. Parathion, methyl parathion, Thimet, Guthion, and Azodrin were administered to rats by the oral, dermal, intravenous or inhalation routes, and the LD_{50}s or LC_{50}s were compared. Inhalation was the most toxic route of administration, followed by the intravenous, oral, and then dermal routes. Females were more sensitive than males to parathion and Thimet by all routes of administration. Azodrin was more toxic to females by the intravenous and oral routes, and Guthion was more toxic to females by dermal application. No correlation was found between mortality and cholinesterase inhibition or blood or liver pesticide content. No gross or histopathological lesions were identified that could be attributed to pesticide treatment. Timed-pregnant rats were exposed to vapors/aerosols of chloroform, ethylene thiourea, Thimet, Bromacil, and Simazine for 1 to 3 hours daily on days 7 through 14 of gestation. No dose-related terata were found in any of the studies.

17. KEY WORDS AND DOCUMENT ANALYSIS

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