ACETAMIDE, DIMETHYLCARBAMYL CHLORIDE, AND RELATED COMPOUNDS

CARCINOGENICITY AND STRUCTURE-ACTIVITY RELATIONSHIPS. OTHER BIOLOGICAL PROPERTIES. METABOLISM. ENVIRONMENTAL SIGNIFICANCE.

David Y. Lai, Ph. D.
Yin-tak Woo, Ph. D.,
Joseph C. Arcos, D. Sc., and
Mary F. Argus, Ph. D.

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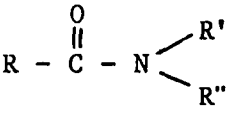
5.2.2.7.1 Introduction.

This group of compounds includes amides of simple carboxylic acids, their N,N-dialkyl derivatives, and related compounds. The general structure of these compounds is:



where R, R', and R" are alkyl groups or hydrogen atoms except in dimethylcarbamyl chloride, where R is a chlorine atom. Adipamide is a diamide derived from adipic acid. The chemical formulas of acetamide and related compounds, which have been bioassayed for carcinogenicity, are shown in Table I.

Early interest in the carcinogenicity of this class of compounds arose from a chance observation in a routine toxicologic study of acetamide. In 1955, Dessau and Jackson, while examining the chronic toxic effects of acetamide, discovered a single hepatic tumor in one rat, together with various hepatic tissue alterations in other animals treated with large doses of acetamide. The weak carcinogenic activity of acetamide was confirmed in subsequent studies (1-3). A number of investigators found acetamide a useful tool for the study of experimental liver cancer because of its low toxicity and simplicity of chemical structure (1, 3).

Because of their homology and/or similarity to acetamide, the structure-activity relationships of several amides and their substituted derivatives have been explored (2, 4). For example, hexaneamide, a higher homolog of acetamide, has been found to induce malignant lymphomas in mice (3). Di-

methylcarbamyl chloride (DMCC), an industrial chemical used as an intermediate in the manufacture of carbamate pesticides and pharmaceuticals, is a potent carcinogen in mice (5, 6), and rats and hamsters (7). In 1976, the National Institute for Occupational Safety and Health (NIOSH) had sent a warning to the industries regarding the potential hazard to workers exposed to DMCC, despite the relatively limited quantities of DMCC produced in the United States (8). In a recent abstract publication, Segal et al. (9) have implied that diethylcarbamyl chloride, a close analog of DMCC, is also a carcinogen in rodents.

5.2.2.7.2 Physical and Chemical Properties, Biological Effects.

5.2.2.7.2.1 PHYSICAL AND CHEMICAL PROPERTIES.

The physical and chemical properties of acetamide and related compounds have been described and discussed by various investigators (10-16). Some physical constants of these compounds are presented in Table I. In general, all simple acid amides (except formamide) are crystalline solids. Their boiling points are considerably higher than those of the respective acids. Lower acid amides such as acetamide are soluble in water. Solubility tends to decrease with increasing molecular weight. N-substitution by methyl or ethyl group(s) lowers the melting point and increases the water solubility of the compounds. The N,N-disubstituted amides, dimethyl- and diethylformamide, and dimethyl- and diethylacetamide, are liquids at room temperature. The high dielectric constants, the electron donor properties, and the ability to form complexes render these compounds remarkably suitable as solvents for a wide range of organic and inorganic compounds (15, 16).

Hydrolysis to the parent carboxylic acids and amines or ammonia is the most general reaction of acid amides. The reaction is accelerated by strong

Table I. Chemical Structures and Some Physical Properties of Acetamide, Dimethylcarbamyl Chloride, and Related Compounds[a]

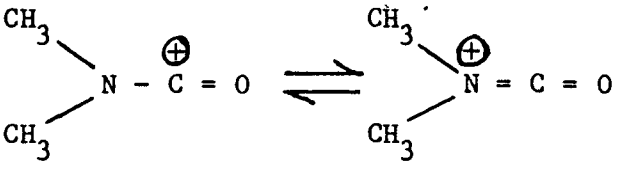
Compound	Formula	b.p. (°C)	Vapor Pressure (mm Hg)	Density (20°C)	Solubility in Water
Acetamide	$CH_3-\overset{O}{\overset{\|\|}{C}}-NH_2$	222	10.0 (105°C)	1.159	98 g/100 ml
Dimethylcarbamyl chloride (DMCC)	$Cl-\overset{O}{\overset{\|\|}{C}}-N(CH_3)_2$	64	—	1.168	Hydrolysis
Dimethylacetamide	$CH_3-\overset{O}{\overset{\|\|}{C}}-N(CH_3)_2$	166	9.0 (60°C)	0.937	Very high[b]
Diethylacetamide	$CH_3-\overset{O}{\overset{\|\|}{C}}-N(C_2H_5)_2$	182	2.0 (35°C)	0.920	--
Dimethylformamide	$H-\overset{O}{\overset{\|\|}{C}}-N(CH_3)_2$	153	3.7 (25°C)	0.953	Very high[b]
Diethylformamide	$H-\overset{O}{\overset{\|\|}{C}}-N(C_2H_5)_2$	177	1.0 (25°C)	0.908	Very high[b]

[a]Data compiled from D.W. Fassett. In: Ind. Hyg. Toxicol. (Patty, F.A., ed.), 2nd ed., Interscience, 1963, p. 1827; and L.J. Fleckenstein. In: Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 2, 2nd ed., 1963, p. 66.

[b]Miscible at any proportion.

acids or bases. For instance, acetamide can be converted to acetic acid by heating with mineral acids or by reaction with nitrous acid. When heated either with phosphorus pentoxide or acetic anhydride, acetamide is dehydrated to yield acetonitrile. Acetamide can also undergo the Hofmann reaction to yield monomethylamine. Reaction of acetamide with alkali metals gives the corresponding metal derivatives in which the metal is linked to the nitrogen. In the presence of hot alkali, acetamide is saponified. Similar reactions occur with disubstituted amides under appropriate conditions.

Dimethylcarbamyl chloride is a colorless liquid prepared by the reaction of dimethylamine with phosgene. The compound may be readily hydrolyzed with a half-life of about 6 minutes at $0^{\circ}C$, yielding dimethylamine, hydrochloric acid and carbon dixoide (17). Dimethylcarbamyl chloride is expected to be a direct-acting acylating agent via its cation (5) which can be stabilized by the resonance structures:



5.2.2.7.2.2 BIOLOGICAL EFFECTS OTHER THAN CARCINOGENESIS

Toxic Effects. Acute studies with different animal species show that acetamide is only slightly toxic; the LD_{50} value by parenteral administration is approximately 10 g/kg in mice and rats (18, 19). In subacute and chronic toxicity studies, acetamide did not induce any significant toxic manifestation in rats and rabbits (18, 19). Early cellular lesions detected microscopically were found to be reversible (18).

Increase in molecular weight and/or N-substitution of the amide with alkyl groups enhances the toxicity of simple amides (18, 20). The LD_{50} values of acetamide and related compounds are given in Table II. Dimethyl and diethylformamide, and dimethyl and diethylacetamide all exhibit similar toxic effects in animals. They are only moderately toxic when ingested or upon brief exposure of the skin to the substance (27-29). Systemic injury can occur when they are inhaled or absorbed through the skin in large quantities over a prolonged period of time. Diethylformamide and diethylacetamide are potent in inducing cardiovascular effects and inhibit the activities of convulsants and hypnotic drugs (30, 31). The clinical symptoms observed in experimental animals administered dimethylformamide or dimethylacetamide chronically include hepatic necrosis, weight loss, anorexia, hyperglycemia, cardiomyopathy, and histopathy in the kidneys, pancreas, spleen, adrenals, and thymus (27, 32-35). Epigastric distress, nausea, vomiting, dermal irritation, abnormal respiratory and hepatic function have also been reported in workers following accidental dermal and respiratory exposure to these compounds (36-39). In addition, alcohol intolerance with flush reaction is a form of adverse response to dimethylformamide exposure in humans (40, 41); this "Antabuse effect" is due to the inhibition of liver alcohol dehydrogenase by dimethylformamide (42). Because of its cytotoxic activity, dimethylacetamide has been used as an experimetnal drug for cancer chemotherapy. Weiss et al. (43) reported that cancer patients receiving high doses of dimethylacetamide developed striking hallucinations with predictable regularity.

Von Hey et al. (21) have undertaken an extensive toxicological study on DMCC. Skin irritation, degeneration of the epidermis, conjunctivitis, and keratitis were seen in rats and rabbits upon contact with undiluted DMCC on the skin and eyes. Inhalation studies in rats showed that almost all animals

Table II. Acute Toxicity of Acetamide, Dimethylcarbamyl Chloride, and Related Compounds

Compound	Species and Route	LD_{50} (g/kg)	Reference
Acetamide	Rat, i.p.	10.30	(18)
	Rat, i.v.	10.00	(19)
	Mouse, i.p.	10.09	(18)
	Mouse, i.v.	8.30	(19)
Dimethylcarbamyl chloride (DMCC)	Rat, oral	1.17	(21)
	Mouse, i.p.	0.35	(21)
n-Hexaneamide	Rat, oral	1.70	(22)
Dimethylacetamide	Rat, oral	5.09	(23)
	Rat, i.v.	2.64	(23)
	Rat, i.p.	3.84	(18)
	Mouse, oral	4.62	(23)
	Mouse, i.v.	2.32	(24)
	Mouse, i.p.	4.19	(18)
Diethylacetamide	Rabbit, i.v.	0.80	(24)
	Rat, i.p.	1.84	(18)
	Mouse, i.p.	1.69	(18)
Dimethylformamide	Rat, oral	2.80	(22)
	Rat, i.v.	2.00	(22)
	Rat, i.p.	1.40	(22)
	Mouse, oral	3.75	(22)
	Mouse, i.v.	2.80	(24)
	Mouse, i.p.	0.65	(22)
	Mouse, inhalation	9.40 (2 hr)[a]	(22)
	Dog, i.v.	0.47	(26)
	Cat, i.p.	0.50	(22)
	Rabbit, i.v.	1.00	(24)
	Rabbit, i.p.	1.00	(22)
	Guinea pig, i.v.	1.03	(22)
Diethylformamide	Rat, i.p.	1.74	(26)
Adipamide	Mouse, oral	6.00	(22)

[a] LC_{50} (gm/m^3)

died after exposure for 1 to 2 hours to an atmosphere saturated at 20°C with DMCC. The chemical caused death by damaging the mucous membrane of the respiratory tract followed by difficulty in breathing. There is also report of eye irritation and liver disturbances in humans exposed to DMCC at the workplace (21).

Mutagenic Effects. Acetamide and DMCC were among the 102 chemicals selected by the U.S. National Cancer Institute for mutagenicity testing in a program to determine the extent of correlation between carcinogenesis and mutagenesis in a standardized assay system (44).

Despite its well established hepatocarcinogenic activity, acetamide is not mutagenic in vitro in various tester strains of Salmonella typhimurium (45-48), Saccharomyces cerevisiae (49), and Escherichia coli (46, 48) tested in vitro with and without microsomal activation or in host-mediated assay (50). Dimethylformamide is inactive in both the Ames test (51) and in the unscheduled DNA synthesis assay using primary rat liver cell cultures (52). No increase in the number of revertants was observed when testing dimethylacetamide in TA1535 and TA100 tester strains of S. typhimurium with or without microsomal activation (53).

In contrast to the amides, dimethylcarbamyl chloride is strongly mutagenic in a series of microbial strains including S. typhimurium TA1535, TA100 (45, 47, 54-56), E. coli WP-2, WP-2S (46, 55) and S. cerevisiae D-3 (49). Diethylcarbamyl chloride is a somewhat weaker mutagen than DMCC and causes base-pair substitutions as well as deletions in S. typhimurium and E. coli (cited in ref. 57). Addition of S-9 mix was not required for mutagenicity in any of these assays.

Teratogenic Effects. A number of studies have indicated that many acid amides and their substituted derivatives are embryotoxic and teratogenic. Acetamide and dimethylacetamide exhibited weak embryolethal effects when they were given by gavage to rabbits between the 6th and 18th day following insemination (58). Moderate embryo mortality was found with dimethylacetamide applied to the skin of pregnant rats during the period of fetal organogenesis (59). Given to rats subcutaneously on day 10 to 14 of pregnancy, dimethylacetamide caused fetal resorption and malformations (59-61). The most sensitive period for the teratogenic effects was found to be around the 10th day of gestation (60). Diethylacetamide was reported to have similar teratogenic activity as dimethylacetamide in the rat (61). Various embryotropic, gonadotropic, and teratogenic effects of dimethylformamide have been repeatedly demonstrated in mice (62, 63), rats (64-66) and rabbits (58, 59) receiving the compound by various routes of administration. These effects include reduced fertility, increased mortality, biochemical changes in the maternal and fetal organs, and embryonal malformations. Regarding humans, Schottek (67) reported that exposure of pregnant women to dimethylformamide resulted in up to 10-fold increase in miscarriage. The compound is believed to penetrate the placental barrier and affect embryonic development (67, 68).

5.2.2.7.3 Carcinogenicity.

Early evidence for the carcinogenicity of acetamide and DMCC have been reviewed in two IARC monographs (69, 70). A summary of the carcinogenicity data of these and related compounds is presented in Table III.

The first report on the carcinogenic activity of acetamide by Dessau and Jackson (71) described a hepatocellular adenoma in 1 of the 5 male Rockland albino rats given oral doses of 4 g/kg acetamide 5 days/week for 205 days.

Table III. Carcinogenicity of Acetamide, Dimethylcarbamyl Chloride and Related Compounds

Compound	Species & Strain	Route	Principal Organs Affected	Reference
Acetamide	Rat, albino, Wistar, or Fischer	oral	Liver	(1-3, 71)
	Mouse, C57Bl/6	oral	Liver, hematopoietic system, stomach	(3)
Dimethylcarbamyl chloride (DMCC)	Mouse, Swiss	topical	Skin, lung	(5, 6)
	Mouse, Swiss	s.c.	Local sarcoma, lung	(5, 6)
	Mouse, Swiss	i.p.	Local sarcoma	(6)
	Hamster, Syrian golden	inhalation	Respiratory tract	(7)
	Rat, --	inhalation	Respiratory tract	(7)
Hexaneamide	Mouse, C57Bl/6	oral	Hematopoietic tissues	(3)
Dimethylacetamide	Rat, Fischer	oral	None	(4)
	Rat, --	inhalation	None	(32)
	Dog, --	inhalation or topical	None	(32)
Dimethylformamide	Rat, Wistar	oral	None	(72)
	Hamster, Syrian golden	i.p.	None	(73)
Adipamide	Mouse, C57Bl/6	oral	None	(3)

Cytological changes related to cell multiplication were also noted in liver cells of other acetamide-treated rats. Later studies from the same laboratory further substantiated the hepatocarcinogenic activity of acetamide (1). In one experiment, fifty 1-month-old male Wistar rats were administered 5% acetamide continuously in the diet. Four of the 48 rats treated for 38-52 weeks developed trabecular carcinomas and adenocarcinomas of the liver, with metastases in the lung. No tumors were noted in the 50 controls. In another experiment, groups of 25 male Wistar rats were continuously fed a diet containing 0, 1.25, 2.5, or 5.0% acetamide for 1 year. Liver tumors, some with invasive growth and distant metastases, were found in 4/24, 6/22, and 1/18 rats treated with low, medium, and high dose levels of acetamide, respectively. None of the 25 control animals developed tumors. When 5% acetamide was fed in the diet to 99 male Wistar rats (and 2 rats were returned to normal diet weekly), liver tumors were seen in 22/81 rats treated for 14-40 weeks. The adenocarcinomas observed in the rats were remarkably similar histologically to human cholangiocellular carcinoma.

These observations were later confirmed by Weisburger et al. (2) who found that 7 of 16 male Wistar rats fed 0.25% acetamide in the diet for 12 months developed malignant liver tumors after a 15-month total observational period. Moreover, Fleischman et al. (3) described neoplastic nodules and hepatocellular carcinomas in rats of both sexes receiving 2.36% acetamide in the diet for 12 months; the tumor incidence was greater in males (41/47) than in females (33/48). The latter study suggests that the occurrence of mixed cell foci and focal fatty changes may represent histopathological markers for the carcinogenicity of acetamide in the rat.

Additional target organs for acetamide carcinogenesis were seen in mice. The study of Fleischman et al. (3) showed that in male mice, there was

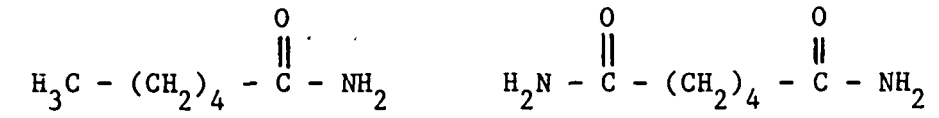
a dose-dependent increase in the incidence of malignant lymphomas. These tumors occurred in 7/50 (14%) and 7/46 (15%) of male mice given 1.18% and 2.36% acetamide, respectively, in the diet for 12 months, compared to 0/95 of the controls. A 5/50 incidence of papillomas in the stomach was also detected in male mice receiving the 1.18% dietary level; however, this was considered to be of questionable significance since it was not observed in male mice receiving the 2.36% dietary level or in any female mice.

Consistent with the negative mutagenic effects of dimethylacetamide and dimethylformamide, a variety of studies failed to demonstrate any carcinogenic activity of these two compounds. In a chronic dermal study by Horn (32), 0.1 or 0.32 ml/kg dimethylacetamide was applied to the skin of 2 dogs of an unspecified strain daily (at each dose level) for 6 months. No neoplastic lesions were observed in the treated animals at the end of exposure. In the study of Hadidian et al. (4) there was no difference in the incidence of neoplastic lesions which occurred in both the control rats and those given 0.1, 3, 10, or 20 mg dimethylacetamide by gavage daily, 5 days/week for a total of 260 doses. Also, no tumors attributable to dimethylacetamide exposure could be detected during a chronic inhalation study in which 2 dogs and 20 rats were exposed to 40, 64.4, 103, or 195 ppm of the compounds for a duration of 6 months on a 6 hour/day, 5 days/week basis (32).

Dimethylformamide has been used as a solvent control in carcinogenicity studies on aflatoxin. No tumors were found in 19 Wistar rats given a single intragastric dose of 0.1 ml of dimethylformamide and observed for 13 to 34 months (72). Neoplastic lesions were observed neither in 10 Syrian hamsters receiving 0.1 ml 50% solution of dimethylformamide weekly for 6-8.5 months by intraperitoneal injection (73). The lack of carcinogenicity of dimethylformamide is further supported by the studies of Purchase et al. (51). These

investigators found dimethylformamide negative in six standard short-term tests for carcinogenicity, while dimethylcarbamyl chloride was positive in four of these assays.

The testing of hexaneamide and adipamide



Hexaneamide Adipamide

for carcinogenicity was carried out in Fischer 344 rats and C57Bl/6 mice by Fleischman et al. (3). Administration of hexaneamide at dietary levels of 1.0% and 1.5% for 12 months did not induce significant carcinogenic effects in rats of either sex, or in female mice. However, 6/35 and 6/39 male mice receiving 1.0% and 1.5% hexaneamide in the diet, respectively, developed malignant lymphomas which were described as grossly and microscopically resembling those induced by acetamide. None of the 95 matched controls bore this type of tumors. Adipamide was given to 50 rats and 50 mice of both sexes in the diet (2.4% and 5.8% in rats; 1.6% and 2.4% in mice) for 12 months. Five of 49 male rats which survived the high dose developed the type of liver tumor induced by acetamide. All the 5 rats bearing neoplasms were housed in the same cage. Because of the unusual distribution of the affected animals, the results were interpreted by the investigators to be inconclusive. No significant incidences of tumors were observed in female rats or mice of both sexes ingesting adipamide at high or low dose.

Dimethylcarbamyl chloride is a potent carcinogen in mice when applied to the skin or injected subcutaneously. In 1972 Van Duuren et al. (5) observed a 60% incidence of skin tumors (46% papillomas and 22% carcinomas) and a 4%

incidence of papillary tumors of the lung in 50 female ICR/Ha Swiss mice following application of 2 mg DMCC to the skin three times weekly for 55 weeks. Subcutaneous injections of 5 mg DMCC in 0.05 ml tricaprylin weekly to 50 female mice of the same strain weekly for 26 weeks resulted in a 72% incidence of local sarcomas and an 8% incidence of papillary tumors of the lung (5). No tumors were noted in the solvent control group or in the untreated groups.

When DMCC (1 mg in 0.05 ml tricaprylin) was applied to mice weekly by intraperitoneal injection for 65 weeks, 8/30 treated mice developed local sarcomas compared with 1/30 control mice given the solvent alone and 0/100 untreated mice (6). Fourteen of 20 DMCC-treated mice, 10 of 30 control mice given tricaprylin alone, and 29 of 100 untreated mice also developed papillary tumors of the lung.

The findings of an inhalation study by Sellakumar et al. (7) using hamsters and rats further emphasize the potent carcinogenic activity of DMCC. In this study, male Syrian golden hamsters and rats of an unspecified strain were exposed to 1 ppm DMCC 6 hours/day, 5 days/week for the lifetime of the animals. The rats were highly sensitive to the compound; 94 of 98 treated animals developed squamous cell carcinomas of the nasal tract 196-348 days after the onset of the exposure (57; cited in ref. 74). Although hamsters are notably resistant to other pulmonary carcinogens (75, 76), 50 of 99 DMCC-exposed hamsters developed tumors in the nasal cavity between day 406 and day 770 of the exposure. No tumors were observed in the 120 untreated or the 50 matched air-exposed controls.

There has been no report on the carcinogenicity study of diethylcarbamyl chloride. However, the similarities in structure and in mutagenic action

between DMCC and diethylcarbamyl chloride have led Van Duuren to the conclusion that diethylcarbamyl chloride has a high probability of being a carcinogen (57). A recent communication by Segal et al. (9) implies that diethylcarbamyl chloride is a rodent carcinogen, with an activity somewhat lower than DMCC.

5.2.2.7.4 Metabolism and Mechanisms of Action.

Metabolism studies in animals showed that 70% of the acetamide administered is excreted unchanged in the urine over a 4-day period (77). However, a small amount of the compound is believed to undergo hydrolysis or enzymatic deamination to yield acetate and ammonium ion (2). The mechanism of carcinogenic action of the compound, despite (or because of) its structural simplicity, is still unknown. Weisburger et al. (2) have shown that arginine glutamate, an agent which counteracts the toxicity of ammonia (78), has a protective effect against the hepatocarcinogenicity of acetamide in rats. The finding led these investigators to the hypothesis that acetamide may be carcinogenic toward the liver because of chronic intracellular liberation of ammonia. That dimethylformamide and dimethylacetamide, with their amide hydrogen atoms replaced by methyl groups, are not carcinogenic (Section 5.2.2.6.3) appeared to be in line with this hypothesis. However, the level of urinary and serum ammonia nitrogen in rats fed acetamide after 4 weeks or 12 months was found not different from that observed in controls (2). Furthermore, feeding of ammonium citrate to rats at dose levels equimolar to a carcinogenic dose of acetamide for 12 months did not elicit any neoplastic lesion in the liver (2). Fleischman et al. (3) suggested that acetamide and hexaneamide might cause lymphomas in mice probably by interaction with latent viruses or endogeneous hormones.

Amides are generally regarded as hydrogen-bonding agents which modify the tertiary structure of macromolecules. Since certain chemical carcinogens alter the conformation of proteins and nucleic acids, it has been hypothesized that carcinogenesis may involve small selective conformational changes or drastic denaturation of certain biological macromolecules essential to the growth and control of target cells (79, 80). In vitro studies have indeed demonstrated that acetamide brings about the denaturation of proteins (80) as well as the formation of hydrogen-bonded associations with nucleic acids (81, 82). Autoradiographic studies by Kaji et al. (83) showed that ^{3}H-acetamide incorporates into the nuclei of Ehrlich ascites tumor cells. Like ^{3}H-thymidine, the incorporation of ^{3}H-acetamide into nuclei was inhibited by mitomycin C, indicating that the incorporation of acetamide is in close connection with DNA synthesis. Based on the observations that acetamide inhibits the incorporation of ^{3}H-thymidine into the nucleotide pool and into DNA, as well as the incorporation of ^{32}P into the phospholipids of cultured human cells, Keysary and Kohn (84) suggested that acetamide may alter the cell membrane. The epigenetic theory for acetamide carcinogenesis would provide an explanation for the negative results in the mutagenicity tests of acetamide (Section 5.2.2.7.2.2). The relationships between effect on DNA synthesis and cell membrane effects, and between denaturation of cellular macromolecules and carcinogenesis by acetamide remain to be investigated.

It has already been pointed out that DMCC is a direct-acting acylating carcinogen* via the cation $(CH_3)_2N\overset{\oplus}{-}C=O$, which is highly reactive toward nucleophiles (5). Recently, the formation of O^6-dimethylcarbamyldeoxyguanosine following *in vitro* reaction between DMCC and calf thymus DNA has been shown (9). Similarly, O^6-diethylcarbamyldeoxyguanosine was detected in the nucleoside hydrolysate following *in vitro* reaction of diethylcarbamyl chloride and DNA (9).

The metabolism of dimethylformamide and dimethylacetamide has been studied both *in vitro* and *in vivo* by Barnes and coworkers (85, 86). *In vitro* studies with rat liver homogenates have indicated that demethylation is the metabolic pathway for these amides. Demethylation was enhanced when the rats were pretreated with phenobarbital, presumably because of the induction of the N-demethylase in the microsomes. N-Monomethylformamide and N-monomethylacetamide have been isolated and identified as the major urinary metabolites in rats administered dimethylformamide and dimethylacetamide, respectively. These metabolites were also detected in the urine of humans exposed to dimethylformamide and dimethylacetamide (88, 89).

5.2.2.7.5 Environmental Significance.

Acetamide does not occur naturally. However, is has been reported that "over-oxidized," spoiled wine contains acetamide (*cited in* ref. 69).

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*The dimethyl carbamyl cation is an example of a recently emerging new class of carcinogens, the direct-acting acylating agents. The fact that alkylating, arylating, and acylating agents can all function as carcinogens indicate that the chemical nature of the xenobiotic molecular moieties attached to key informational macromolecules is probably immaterial as long as these attachments interfere with the biosynthetic template function of these macromolecules (87). Direct-acting acylating agents will be further discussed in Appendix I of this volume.

Acetamide has recently been identified as a metabolite of metronidazole, a drug used in the treatment of trichomonal vaginitis and various forms of amebiasis (90, 91). Metronidazole was found by Rustia and Shubik (92) to induce lung tumors and malignant lymphomas by oral administration to Swiss mice. However, the carcinogenicity of metronidazole must be attributed to its structural relationship to the 5-nitrofuran carcinogens (see Section 5.1.2.4.1.3, Vol. IIB) rather than to the metabolic release of acetamide.

Since acetamide has numerous industrial uses, human exposure to the compound also occurs in the workplace. Acetamide has been produced commercially in the United States since 1921. In 1978, about 228 metric tons of acetamide was produced (14). It is widely used as solvent, solubilizer, plasticizer, stabilizer, wetting agent, and antacid in the lacquer, explosives, and cosmetics industries (11, 14). In addition, it has been reported to be employed in cryoscopy, in soldering, and in the synthesis of other organic chemicals, insecticides, and hypnotics (11, 14). Because of its low acute toxicity and the lack of awareness of its potential chronic effects, usual handling and use of acetamide were considered in the past to represent no significant hazard.

Acetamide has high water solubility and low vapor pressure. Thus, should it be released in the environment, it will readily enter the soil and the water table. However, unless large amounts are involved, it will not persist and bioaccumulate. Biological tests with bacteria, algae, and fish indicate that very high levels of acetamide are tolerated (93).

Dimethylcarbamyl chloride is not in extensive use and is produced only in limited quantities in the United States. In 1975 only about 3,000 pounds of the compound was manufactured for the synthesis of certain carbamate pesti-

cides and drugs for the treatment of myasthenia gravis (8). Less than 200 persons were estimated to be occupationally exposed to DMCC (8). Diethylcarbamyl chloride, on the other hand, was reported to be in more extensive use in the United States (57). The compound is particularly important in the production of a veterinary antifilarial drug, diethylcarbamazine citrate (57). Because of the carcinogenic properties of DMCC and diethylcarbamyl chloride, industries in the United States have been alerted to the potential hazards involved in handling these chemicals. In the air of a manufacturing plant in the Federal Republic of Germany (cited in ref. 70), concentrations of up to 1.5 ppm DMCC have been reported. However, no cancer deaths or indications of lung cancer were found in an investigation of 65 DMCC workers and 42 ex-workers aged 17-65 and exposed for periods from 6 months to 12 years (cited in ref. 70).

Little information is available on the environmental occurrence or fate of DMCC and diethylcarbamyl chloride. Munn (94) pointed out that DMCC is much less volatile than bis(chloromethyl)ether, a potent human carcinogen known to cause respiratory cancers. Since DMCC is rapidly hydrolyzed in water with a half-life of about 6 minutes at $0^{\circ}C$ (17), the compound will not persist or bioaccumulate in the aquatic environment.

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Notes Added After Completion of Section 5.2.2.7

Dimethylformamide was reported in 1979 to induce chromosomal aberrations in human lymphocytes in vitro (1). However, a more complete study using various test systems did not confirm the genotoxicity of dimethylformamide (2).

In addition to O^6-dimethylcarbamyldeoxyguanosine, 6-dimethylamino-2'-deoxyguanosine and 4-dimethylamino-thymidine are also formed in the in vitro reaction of dimethylcarbamyl chloride with calf thymus DNA (3).

Acrylamide ($CH_2=CH-\overset{O}{\overset{\|}{C}}-NH_2$), a chemical widely used in the synthesis of polymers, bears a structural resemblance to acetamide with respect to the acid amide group, and to two other carcinogens, vinyl carbamate (see Section 5.2.1.6 of Vol. IIIA) and acrylonitrile (see Section 5.2.1.7.2 of Vol. IIIA) with respect to the carbon-carbon double bond. Like acetamide, acrylamide does not produce point mutations in Salmonella typhimurium (4). However, it induces high frequency of chromosomal aberrations in bone marrow and germ cells of mice (5). In 1984, acrylamide was shown to have tumor initiator activity in the skin of Sencar mice, either by topical application or by systemic routes of administration. In addition, the compound induces lung adenomas in strain A/J mice after oral or intraperitoneal administration (4). These findings indicate that acrylamide possesses carcinogenic properties similar to vinyl carbamate and its postulated parent compound, ethyl carbamate (see Section 5.2.1.6 of Vol. IIIA). In fact, acrylamide is as potent as ethyl carbamate in the initiation of mouse skin tumors (4). The mechanisms of tumorigenic action of acrylamide is unknown. It is possible that acrylamide, vinyl carbamate and acrylonitrile all act via similar mechanisms by virtue of the carbon-carbon double bond in their molecules. Acrylamide has been shown to alkylate proteins at the sulfhydryl group (6).

References for Section 5.2.2.7 Update

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