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NITROSAMINES AND PESTICIDES: A SPECIAL REPORT ON THE OCCURRENCE OF NITROSAMINES AS TERMINAL RESIDUES RESULTING FROM AGRICULTURAL USE OF CERTAIN PESTICIDES

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NITROSAMINES AND PESTICIDES
A SPECIAL REPORT ON THE OCCURRENCE OF NITROSAMINES AS TERMINAL RESIDUES RESULTING FROM AGRICULTURAL USE OF CERTAIN PESTICIDES

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ABSTRACT - Nitrosamine contaminants in certain pesticide formulations, especially dinitroaniline and some acid herbicides, has been firmly established. These contaminants arise from either chemical synthesis, as with the dinitroanilines, or from nitrosation in the product due to nitrite, as with certain halobenzoic acid formulations. The nitrosamines are either simple nitrosodialkylamine compounds or the nitroso derivative of the parent pesticides. Methodology for detecting and determining trace levels for both volatile and non-volatile nitrosamines is available. Formation of nitrosated pesticides in soil or water would not appear to be a major problem. In vivo nitrosation of pesticides and dialkylamines has been studied. Concurrent administration of certain amines and nitrite to laboratory animals has resulted in increased tumor information. Trace amounts of nitrosated carbamates and low yields of nitrosated dialkylamines were observed as a result of simultaneously feeding rats and guinea pigs the parent compound and nitrite. No evidence of nitrosoatrazine in stomach contents, tissues, and milk were found when atrazine and nitrite were fed to rats and goats. Nitrosamines are photolabile and the volatile members of the class are partially dissipated by volatilization and subsequent photodecomposition in air. Most nitrosamines are stable to hydrolysis in aqueous solution at the pH's usually found in ground and surface waters and are photodegraded in solutions. The nitrosodialkylamines are rapidly dissipated in soil, whereas certain nitrosated pesticides are more stable. Uptake of radioactivity into plants has been demonstrated using ^{14}C-labeled nitroso compounds; however, no nitrosated products have been identified in either the forage or grain of the crops studied.

INTRODUCTION

Nitrosamines, a class of compounds of which several members are known carcinogens in laboratory animals, have recently been reported to be present in certain pesticide formulations (Ross et al., 1977; Bontoyan et al., 1979). Nitrosodialkylamines have been detected in dinitroaniline herbicide formulations and apparently result from the nitrosation of the respective amine during manufacturing. Certain secondary amine dinitroanilines are also nitrosated during manufacturing. Formulations of amine salts of some acidic herbicides have also been reported to contain nitroso derivatives of the respective amines.

Nitrosamine contamination of the amine used for formulation and nitrosation of the amine by nitrite present as a corrosion inhibitor are the most probable sources. The levels of the nitrosamines found have ranged up to 640 ppm. The tentative identification of a nitroso derivative of the herbicide atrazine in soil and water has also been reported (Fine et al., 1975). Although many of the nitrosamines studied are suspect human carcinogens, their carcinogenic potential in man has not been established.

Since the original reports on nitrosamines associated with pesticides, improvements in pesticide manufacturing processes and formulations have reduced the concentration of nitrosamine in most pesticide formulations. Extensive monitoring studies

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have also been conducted on environmental residues resulting from nitrosamine impurities in pesticide formulations. Human exposure from these sources is negligible. There is sufficient international scientific interest in nitrosamines in pesticides to merit an indepth review of the problem by the Commission on Terminal Pesticide Residues, Applied Chemistry Division, International Union of Pure and Applied Chemistry, as part of its annual meeting in Deidesheim, West Germany, July 17-21, 1978. The results of the review are contained in this special report.

The scope of this review is limited to the chemistry of nitrosamines associated with pesticides. Several comprehensive reviews on the chemistry and toxicology of nitrosamines are available (Magee et al., 1976; Mirvish 1975, Douglass et al., 1978).

1. CHEMISTRY

1.1 Nitrosamines and Nitrosamides

N-Nitroso compounds are conveniently divided into two fairly distinct categories, nitrosamines and nitrosamides. The nitrosamine (category one) consists of compounds of the general structure $R^1R^2N\text{-}N\text{=}O$ where R^1 and R^2 can be alkyl or aryl groups, or parts of a ring. The nitrosamides (category two) are more diverse and less accurately named, but consist primarily of compounds with a carbon heteroatom double bond adjacent to the nitrogen, RN(NO)CY. Most frequently X=O as with nitrosamides (X=O, Y=alkyl, aryl), nitrosocarbamates (X=O, Y=OR^1), and nitrosoureas (X=O, Y=NH_2, NHR, NR_2, etc.). For convenience, nitrosoguanidines (X and Y=NH and/or NR) can also be included in this second category, along with nitrososulfonamides ($RN(NO)SO_2R^1$). Since pesticides encompass all of these classes, the variety of possible nitrosated pesticides is considerable.

1.2. Properties

A detailed listing of the chemical and physical properties of many nitrosamines has been summarized (IARC, 1978). Some general properties are discussed in this section. The lower molecular weight analogs may be oils or solids. A few can be distilled at atmospheric pressure, and several others can be distilled at reduced pressure. The nitrosamines are hydrolyzed to the precursor amines with strong acids (Preussmann et al., 1972). Steam distillations from sodium or potassium hydroxide solutions, or from mildly acidic solutions, constitute common cleanup procedures for extracting steam-volatile nitrosamines from food stuffs, biological materials, and soils.

Reports on the photolability of nitrosamines vary; evidently they are rapidly photodecomposed (with initial cleavage of the N-N bond) in dilute acid solutions, but are relatively stable to light in neutral solutions (Chow, 1967). Other reports (Burns and Alliston, 1971; Polo and Chow, 1976) indicate nitrosamines are photolabile in aqueous solutions (pH 7-10) but are more readily photolyzed at lower pH values. There are apparently no pH effects in the ranges (pH 3-9) normally observed for natural waters (Saunders and Mosier, 1979). Although data are scarce, it is generally believed that nitrosamines are rapidly decomposed by light in the vapor phase (Bamford, 1939; Hanst et al., 1977, Pitts, 1978). The half-life of NDPA in air is estimated to be about 20 minutes under cloudy conditions and 10 minutes in bright sunlight (Mazzocchi, 1979). In addition, nitrosamines are subject to oxidation, reduction, alkylation, condensation, and other types of reactions with appropriate reagents. Some of these provide useful analytical procedures and will be discussed in that context, but most, except possibly photooxidation to N-nitramines, do not seem to be of great environmental significance.

Nitrosamides are yellow or yellow-orange oils or solids; in contrast to nitrosamines they are thermally labile and tend to rearrange to diazoesters. The diazoesters are also unstable and decompose to carbonium ions if the amides were derived from aliphatic amines, or to free radicals if derived from aromatic amines (White, 1955). Nitrosamides are very sensitive to alkaline conditions which catalyze degradation to diazoalkanes or to diazonium ions. The former reaction is the basis for the laboratory preparations of diazomethane from any of several familiar reagents. Like the nitrosamines, nitrosamides are cleaved back to the parent amides with strong acids (Sander et al., 1971) and are in fact more easily hydrolyzed by mild acids than are nitrosamines (Singer et al., 1977). Like nitrosamines, nitrosamides are decomposed by photolysis of weakly acidic solutions (Chow and Lee, 1967). Nitrosamides also subject to photodecomposition in neutral solutions, although more slowly than in acid solutions (Chow and Lee, 1967).

1.3. Preparation

Classically, nitrosamines are prepared from secondary amines and aqueous solutions of nitrous acid, HNO_2 (from the acidification of sodium nitrite), but a variety of other nitrosating agents and solvents can be used as well (Mirvish, 1975). Many more stable nitrosamides can also be formed from aqueous nitrous acid, but dinitrogen tetroxide in anhydrous solvents has been shown to be a superior nitrosating medium (White, 1955). This same system is also useful for the nitrosation of relatively unreactive or poorly soluble amines, like atrazine (Kearney et al., 1977) and butralin (Oliver and Kontson, 1978).

In addition to secondary amines, which form nitrosamines directly, nitrosations of primary and tertiary amines, and even of quaternary ammonium salts, are possible. Primary amines nitrosate rapidly, but the nitrosamines are unstable and spontaneously dehydrate to diazonium ions. The diazonium ions are also unstable, particularly those formed from aliphatic amines, and decompose usually with loss of nitrogen. Diazonium ions from aromatic amines, however, can react with primary or secondary amines to form triazenes, some of which are also carcinogens (Magee et al., 1976).

Tertiary amines can also form nitrosodialkylamines upon reaction with nitrous acid (Mirvish, 1972). An initial oxidative dealkylation produces a secondary amine, which is then nitrosated.

2. DETERMINATION

2.1. Colorimetric Methods

Daiber and Preussmann (1964) have developed methods for the colorimetric determination of nitrosamines and nitrosamides. Under UV irradiation, these compounds produce nitrous acid, either quantitatively (nitrosamines) or with fair to poor yields (nitrosamides). The nitrous acid is trapped in aqueous sodium carbonate and determined with the well-known Griess-Jlosvay reagent (sulfanilic acid/1-naphthylamine). Sensitivity is about 1-2 µg/ml. Keller and Drescher (1974) have developed a similar method for determination of alkyl-N-nitrosohydroxyamines in milk with a detection limit of ca. 0.05 mg/kg. An improved colorimetric method has been published by Eisenbrand and Preussmann (1970). The nitrosamine is reacted with HBr/glacial acetic acid to form nitrosylbromide (BrNO) and the corresponding amine hydrobromide. The liberated BrNO is reacted with sulfanilic acid and the resulting diazonium derivative is coupled with N-(1-naphthyl)-ethylenediamine. The sensitivity of the method is calculated to be 2-3 µg/kg (using a 1 kg/sample). Alternatively, the liberated amine can be reacted with heptafluorobutyryl chloride and the derivative determined by gas chromatography (Eisenbrand, 1972).

The lower nitrosamines (dimethyl-, methyl, ethyl, and diethyl-) gave quantitative results with the hydrazine method of Ender and Ceh (1971). By reaction with Zn/HCl, the nitrosamine is reduced to the corresponding asymmetric hydrazine, which is derivatized with 4-dimethylaminobenzaldehyde to form a yellow aldazine. A lower limit of determinization of 0.001-0.01 mg/kg is reported.

Colorimetry as a screening method for the direct estimation of total non-volatile nitrosamines, without their extraction from the matrix, has been proposed (Walters et al., 1974). Thionyl chloride is used as the denitrosating agent. The liberated volatile ClNO is reacted with sulfanilamide and N-(1-naphthyl) ethylenediamine for color development.

Recently, Chuong and Benarie (1976) have described a method for determination of nitrosamines in air in the presence of NO_2. The NO_2 is separated and adsorbed by bubbling the air through Griess-Salzmann reagent. Nitrosamines, before colorimetric determination, are trapped by selective filtration on a microporous membrane.

2.2 Gas Chromatography

Many gas chromatographic methods have been developed for determination of nitrosamines using a wide variety of very non-polar to highly polar packed and capillary columns. Detectors used for quantitative determination include FID, AFID, EC, MS, Coulometric and Coulson-detector. Nitrosamines are determined either directly or after derivatization e.g., oxidation to the corresponding N-nitramines, splitting off the nitroso group, and derivatization of the secondary amine formed. Table I gives some examples of GC methods available together with appropriate references. As can be seen from Table I, most of the methods focus on the volatile, low molecular weight aliphatic and cyclic nitrosamines and very few methods are available for non-volatile nitroso compounds.

2.3 Thermal Energy Analyzer

A new analytical instrument highly specific and extremely sensitive for both volatile and non-volatile N-nitroso compounds, has been developed by Fine et al., (1973) and Fine and Rufeh (1974). The apparatus, called a Thermal Energy Analyzer (TEA) or Thermoluminous Analyzer, operates as follows: The N-nitroso compound, usually dissolved in dichloromethane, is injected into a flash catalytic pyrolyzer, where the N-NO bonds are ruptured to form nitrosyl radicals ($NO^{\bullet}$), which are swept into a connected reaction chamber. Ozone, developed by electric discharge, also enters the chamber and reacts with the nitrosyl radicals giving excited NO_2^*. The excited molecules rapidly decay to their ground state with characteristic emission in the near infrared. The light emission is measured with an IR-sensitive photomultiplier response, which is directly proportional to the number of decays and thus to the number of moles of the N-nitroso compound, is amplified and displayed on a chart recorder.

A more detailed description of the TEA and a discussion of its theoretical basis has been given by Fine et al., (1975), as well as by Glover (1975). The detector has been shown to be linear over four to six orders of magnitude (Fine and Rufeh, 1974).

TABLE 1. GAS CHROMATOGRAPHY

Detector	NO-amines	Derivative examined	Substrate (fortification level)	Limit of detection	Recovery %	Reference
FID confirmation by MS	dimethyl	-	herring meal	0.1 mg/kg	-	Hurst (1976)
AFID (KCl)	9 volatile	-	meat) fish) (10 and 20 µg/kg)		70 - 114	Fazio et al. (1972)
AFID (KCl)	dimethyl	-	smoked fish (10 µ/kg)	0.01 mg/kg	70 - 866	Howard et al. (1970)
AFID (Rb_2SO_4)	dimethyl	-	cooked and smoked ham canned ham (25 µg/kg)	0.025 mg/kg	79 - 92 73 - 100	Fiddler et al. (1971)
AFID (Rb_2SO_4)	dimethyl	-	soil) grains) spinach) lettuce) (4 - 16 µg/kg) carrots) tomatoes)	4 - 8 µg/kg	75 - 85	Dressel (1976)
AFID (RbBr)	6 volatile	-	natural water waste water (10 µg/kg) sludge	0.1 µg/kg	46 - 93 pyrrolidine 15 methylaniline 27	Dure et al. (1975)
EC	dimethyl	Dimethylnitramine (after oxidation with $F_3C-COOH/H_2O_2$)	smoked hake	16 pg	-	Sen (1970)

TABLE 1. Continued

Detector	NO-amines	Derivative examined	Substrate (fortification level)	Limit of detection	Recovery %	Reference
EC and FID	10 volatile	corresponding nitramines (after oxidation with F_3C-COOOH)	none	-	-	Althorpe et al. (1970)
EC (confirmation by MS C_3F_7-fragment	11 volatile	corresponding hepta-fluorobutyrylamines (after HBr/HAc denitrosation and reaction with C_3F_7COCl)	wheat flour (dimethyl, diethyl-diamyl only) (22 and 34 µg/kg)	<0.02 mg/kg	62 - 82	Eisenbrand (1972)
EC	6 volatile	corresponding hepta-fluorobutyrylamides after electrochemical reduction & reaction with C_3F_7COCl	cheese meat fish fat	1 pg/kg	35 - 86	Alliston et al. (1972)
FID and MS	amino acids sarcosine proline 2-hydroxy-proline	trimethylsilyl-NO-amino-acids	none	1 ng	-	Eisenbrand et al. (1975)
MS	dimethyl pyrrolidine	-	cooked bacon cooked out fat vapour	<0.01 mg/kg	-	Gough et al. (1976)
MS	dimethyl diethyl di-n-butyl pyrrolidine piperidine	-	various meat products (10 - 50 µg/kg)	0.1 - 0.2 µg/kg	70 - 103 pyrrolidine: 40 - 60	Stephany et al. (1976)

TABLE 1. Continued

Detector	NO-amines	Derivative examined	Substrate (fortification level)	Limit of detection	Recovery %	Reference
MS	16 volatile	-	meat (1 - 40 µg/kg)	1 µg/kg	31 - 92	Osbourne (1972)
MS	25 volatile	-	-	0.01 - 0.5 µg	-	Heyns et al. (1970)
Micro-coulometer	dimethyl-	NH_3	raw and cooked apples milk (10 µ/kg)	3 µg/kg	>70	Newell et al (1972)
Coulson	dimethyl- (from Ziram Ferbam and dipyrrolidyl-thiuram- di-sulphide)	NH_3	articial gastric juice (10^{-4} moles)		95 - 97	Eisenbrand et al. (1974) -
EC and Coulson	8 volatile	corresponding nitramines after $F_3C-COOH/H_2O_2$-oxidation NH_3	bread (10 µg/kg)	5 µg/kg	>60	Walker et al. (1975)

Several hundred compounds of different chemical structure (from inorganic gases up to dyestuffs and pharmaceuticals) have been tested and found not to interfere with the determination of N-nitroso compounds. There are, however, some chemicals, other than nitrosamines, that cause a response in the TEA, e.g., 2,2',4,4'6,6'-hexanitrodiphenylamine (molar response ratio RR = 1.4); pentyl- and isopentylnitrite (RR = 1.0), aqueous solutions of sodium nitrite, nitrate, and nitric acid (RR = 1). Positive responses of lower orders of magnitude have been observed, e.g., with dimethyl sulfoxide, hydrazine (RR = 0.03), 4-nitrosodiphenylamine (RR = 0.005), nitromethane (RR = 0.0018), and aniline (RR = 0.0003). (The very small responses, however, may be caused by impurities, rather than by the chemical.)

Samples can be injected directly into the TEA (TEA-DI mode) or the TEA may be used as the detector for an externally interfaced gas chromatograph (TEA/GLC mode) or high pressure liquid chromatograph (TEA/HPLC mode). In the latter modes, it is possible to distinguish between volatile (TEA/GC) or non-volatile (TEA/HPLC) nitrosamines. Recoveries of nitrosodimethylamine (NDMA) added (20-242 µg/kg) to fresh beef and fresh herring and analyzed with the TEA/DI were 75-97%, whereas recovery of the non-volatile nitrosodiphenylamine from spiked herring (60 and 1270 µg/kg) was 100 and 75%, respectively (Fine and Rufeh, 1974). Fine and Rounbeheler (1975) have analyzed standard solutions of several nitrosated amines (dimethylamine, diethylamine, dipropylamine, dibutylamine, piperidine, pyrolidine and sarcosine) by means of the TEA/GC in the sub-level without concentration or extensive clean-up. Fine et al., (1975) used the TEA/GC-combination to determine volatile nitrosamines in canned tuna fish, canned beef and soybean oil. Recoveries of 71-100% were obtained. The sensitivity of the method was at the 5 µg/kg level without concentration and a 100-fold higher sensitivity after concentration of the extract was expected to be attainable.

Both TEA/GC and TEA/HPLC were used to determine NDMA in air samples and NDMA, as well as nitrosodipropylamine (NDPA) in pesticide formulations, air and water (Fine and Ross, 1976), TEA/GC methods have been developed for analyses of volatile nitrosamine contaminates in formulated and technical dinitroaniline herbicides (Day et al., in press) and for volatile nitrosamines in crops and soils treated with dinitroaniline herbicides (West and Day, in press). Both methods included column chromatography on alumina before TEA/GC analysis.

Positive results obtained with the TEA should be evaluated very critically and, whenever possible, be confirmed by an independent specific method, like GC/MS.

2.4. Other Methods

2.4.1 Thin-layer chromatography has been applied by Sen and Dalphe (1972) for semiquantitative determination of volatile nitrosamines, using Griess and/or ninhydrin reagent for visualization. The sensitivity of the Griess detection method can be increased substantially (Ohnsorge and Drescher, 1975), by spraying the plate with a 1% aqueous solution of sodium sulfanilate, exposing the plate to conc. HCl vapor and spraying the acidified layer with 0.1% aqueous solution of N-(1-naphthyl)-ethylendiamine. Especially acid-sensitive nitrosamines may be chromatographed on silica gel G plates prepared using N $NaHCO_3$, instead of water or, alternately, on prefabricated silica gel HF_{254} plates that have been dipped in 1 N $NaHCO_3$ and air dried (Ohnsorge and Drescher, 1975). A quantitative TLC method for NDMA, NDEA, and N-nitroso-di-n-amylamine nitrosamines has been described by Eisenbrand et al., (1970). The procedure also can be used as a cleanup step, when the respective zones are scraped out from the plate and the nitrosamines are liberated by steam distillation.

2.4.2 High pressure liquid chromatography was used by Klimisch and Ambrosius (1967) for quantitative determination of several volatile N-nitrosamines. The compounds, after denitrosation, were reacted with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) to form the respective NBD-amines. Twenty-five nonograms and 0.5 ng of the NBC-amines were detectable with an UV and fluorescense-detector, respectively. Eisenbrand et al., (1970) found an efficient separation of three unsymmetrical and six symmetrical aliphatic N-nitrosamines by gel chromatography on Sephadex LH 20. The method, however, failed when biological material (wheat flour extract) was present due to interferences in the UV detector.

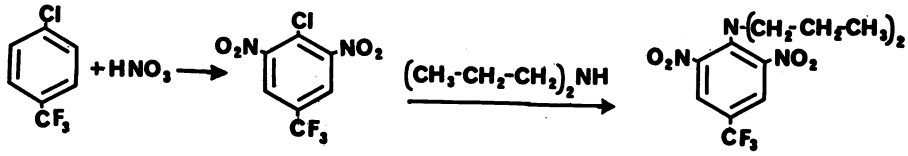
2.4.3 A liquid chromatographic method, using a strong cation exchange column and acetonitrile/water as the mobile phase has been developed by Wolfe and coworkers (1976) to study the formation and photodegradation of nitrosoatrazine in water. The method has a sensitivity of 1 mg/kg.

2.4.4 Differential polarography at low pH has been applied for the determination of volatile and non-volatile nitrosamines by Walters et al. (1970) and a procedure for separation and detection of these compounds in biological material is recommended.

3. FORMATION

3.1. Manufacturing

The presence of nitrosamine contaminants in the dinitroaniline herbicides is reported to result from nitrosation of the respective amine used in the synthesis of the herbicide (Ross et al., 1977) or from nitrosation of the pesticide itself during chemical synthesis. The presence of NDPA in trifluralin is speculated to result from nitrosation of dipropylamine during amination of residual oxides of nitrogen present in the reaction mixture from a previous nitration step (Figure 1).



$$HNO_3 + \text{EXCESS}\ \ (CH_3\text{-}CH_2\text{-}CH_2)_2NH \longrightarrow (CH_3CH_2CH_2)_2N\text{-}NO$$

Fig. 1. Formation of NDPA during trifluralin synthesis.

The discovery of nitrosamines (Bontoyan et al., 1979; Pest. Toxic, 1977; Cohen et al., 1978) in other dinitroaniline herbicides manufactured by processes similar to those used for trifluralin is supportive of the suspected mode of nitrosamine formation. Some dinitroanilines seem to be relatively free of nitrosamines. In these cases, the manufacturing processes may be different enough so that the nitrosamines are not formed or, if they are formed, they are subsequently destroyed in the synthetic sequence.

Certain dinitroanilines which are secondary amines, such as butralin or pendimethalin (Cohen et al., 1977; Bontoyan et al., 1979), are reported to contain contaminant levels of the nitroso derivative of the parent molecule. In the synthesis of pendimethalin, the nitration step is performed on an aniline, and the final synthesis step is a denitrosation with sulfamic acid (Levy et al., 1975), which apparently is not totally effective in removing the nitrosated species. Recently, Eizember (1978) and Cannon and Eizember (1978) have described procedures to reduce the levels of nitrosamines formed during dinitroaniline manufacture. The first involves treatment of the final product with halogenating agents, like bromine, chlorine, or N-bromosuccinimide. The second entails base (e.g., sodium carbonate) treatment and aeration of the nitration product, 1-chloro-2,6-dinitro-4-(trifluoromethyl) benzene (Figure 1), to remove the nitrogen oxides that are presumably responsible for the nitrosation of dipropylamine in the final step of trifluralin synthesis.

3.2. Formulation

A second category of nitrosamines associated with commercial pesticides has been in acidic products (e.g., substituted phenoxy or benzoic acids formulated as amine salts, Ross et al., 1977; Pest. Toxic. 1977; Cohen et al., 1978). These nitrosamines, where present, have corresponded to the secondary amines (usually dimethylamine or diethanolamine) used in the formulation. High concentrations of NDMA (up to 640 ppm) were detected in formulated 2,3,6-trichlorobenzoic acid stored in metal containers where sodium nitrite had been used as a corrosion inhibitor, and it has generally been assumed (Ross et al., 1977) that the amines reacted with a nitrosating species formed in situ. The situation is complicated, however, by the detection of NDMA in some batches of dimethylamine used for formulations (Pest. Toxic, 1977; Cohen et al., 1978). The former problem will presumably be solved by using alternate corrosion inhibitors, but the status of the second is less certain at this point.

3.3 Laboratory Synthesis

Under laboratory conditions, several pesticides have converted to their nitroso derivatives for a variety of purposes, including toxicological testing. A listing of many of these nitrosated pesticides is shown in Table 2.

Table 2. Pesticides Nitrosated Under Laboratory Conditions

Common Name	Chemical Name	Reference
Acephate	0,S-dimethyl-N-acetyl-phosphoramidothioate	Seiler, 1977
Aldicarb	2-methyl-2-(methylthio)-propionaldehyde-o-(methyl-carbamoyl)oxime	" "
Antu	1-napthylthiourea	" "
Atrazine	2-chloro-4-ethylamino)-6-(isopropyl)-s-triazine	Eisenbrand et al., 1975 Kearney et al., 1977
Bassa	2-sec-butylphenyl-N-methylcarbamate	Uchiyama et al., 1975
Benomyl	methyl-1-(butylcarbamoyl)-benzimidazole-2-yl-carbamate	Seiler, 1977
Benzthiazuron	1-(2-benzothiazolyl)-3-methylurea	Eisenbrand et al., 1977 Seiler, 1977
Butralin	4-(1,1-dimethylethyl)-N-(1-methylpropyl)-2,6-dinitrobenzenamine	Oliver and Kontson, 1978
Buturon	3-(4-chlorophenyl-1-methyl-1-(1-methylprop-2-ynyl)urea	Seiler, 1977
Carbaryl	1-napthyl-methylcarbamate	Egert and Greim, 1976 Eisenbrand et al., 1975 Elispuru et al., 1974 Seiler, 1977 Uchiyama et al., 1975
Carbendazin	methyl-2-benzimidazole carbamate	Seiler, 1977
Carbofuran	2,3-dihydro-2,2-dimethylbenzofuran-7yl-methylcarbamate	" "
Chlorobromuron	3-(4-bromo-3-chlorophenyl-1-methoxy-1-methylurea	" "
Chlorothiamid	2,6-dichlorothiobenzamide	" "
Chlorotoluron	3-(3-chloro-p-tolyl)-1,1-dimethylurea	" "
Chloroxuron	3[p-(p-chlorophenoxy)phenyl]-1,1-dimethylurea	Egert and Greim, 1976

Table 2. Continued

Common Name	Chemical Name	Reference
Cychuron	3-cycloocty1-1,1-dimethylurea	Egert and Greim, 1976
Cypendazole	methyl 1-(5-	Seiller, 1977
Daminozide	succinic acid 2,2-dimethylhydrazide	" "
Dimethoate	O,O-dimethyl-S-(N-methylcarbamoylmethyl) phosphorodithioate	" "
Dimefox	N,N,N',N'-teramethylphosphorodiamidic fluoride	Egert and Greim, 1976
Dioxacarb	O-1,3-dioxolan-2-ylphenyl methylcarbamate	Seiler, 1977
Diuron	3-(3,4-dichlorophenyl)-1,1-dimethylurea	Seiler, 1977
Dodine	dodecylguanidine acetate	" "
Ethiofencarb	2-ethylthiomethylphenyl methylcarbamate	Egert and Greim, 1978 Seiler, 1977
ETU	ethylenethiourea	" "
Ferbam	ferric dimethyldithiocarbamate	Sen et al., 1974
Fluometuron	1,1-dimethyl-3-(3-trifluoromethyl)phenylurea	Seiler, 1977
Formelanate	3-(dimethylamino)methylene-aminophenyl methylcarbamate	" "
Glyphosate	N-(phosphonomethyl) glycine	" " Khan and Young, 1977
Hopcide	2-chlorophenyl-N-methylcarbamate	Uchiyama et al., 1975
Linuron	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea	Seiler, 1977
Maqbarl	3,5-xylyl N-methylcarbamate	" "
Meobal	3,4-xylyl N-methylcarbamate	" "

Table 2. Continued

Common Name	Chemical Name	Reference
Methabenzthiazuron	1-(2-benzothiazolyl)-1,3-dimethylurea	Uchiyama et al., 1975
Methomyl	S-methyl-N-(methylcarbamoyl-oxy)-thioacetimidate	Seiler, 1977
Metoxuron	3-(3-chloro-4-methoxyphenyl)-1,1-dimethylurea	" "
Mipsin	2-isopropylphenyl N-methylcarbamate	" "
Monolinuron	3(p-chlorophenyl)-1-methoxy-1-methylurea	" "
Monuron	3(p-chlorophenyl)-1,1-dimethylurea	" "
Phenmedipham	3-metoxycarbonylaminophenyl N-(3-methylphenyl)carbamate	" "
Prometryne	2,4 bis(isopropylamino)-6-(methylthio)-3-triazine	Egert and Greim, 1976
Propham	isopropylcarbanilate	Seiler, 1977
Propoxur	O-isopropoxyphenyl methylcarbamate	Eisenbrand et al., 1975
Propyzamid	N-(1,1-dimethylpropynyl)-3,5-dichlorobenzamide	Seiler, 1977
Simazine	2-chloro-4,6-bix(ethylamino)-3-triazine	Eisenbrand, et al., 1975
Suncide	2-isopropoxyphenyl N-methylcarbamate	Seiler, 1977
Swep	methyl-N-(3,4-dichlorophenyl) carbamate	" "
Thiram	bis(dimethylthiocarbamoyl)disulfide	Egert and Greim, 1976 Sen et al., 1975
Tsumacide	3-tolyl N-methylcarbamate	Seiler, 1977
Ziram	zinc dimethyldithiocarbamate	Eisenbrand et al,, 1975

3.4. Environmental Formation

The environmental formation of nitrosamines in systems other than animals has received limited attention. Concern about the possible nitrosation of certain pesticides in soil, water, air and plants has prompted recent activity on the environmental formation of nitrosamines from synthetic amines, including pesticides.

3.4.1. Formation in Soil, Water and Air

In soils, nitrosamines can arise from the parent pesticide or from a pesticide metabolite, like dimethylamine. The capabilities of soil microorganisms to promote, either directly or indirectly, nitrosations have not been fully defined. Verstraete and Alexander (1971) found that NDMA could be generated in raw, but not in autoclaved, sewage amended with diemethylamine, and Ayanaba and Alexander (1973) demonstrated that an extract from Cryptococcus sp. catalyzed nitrosamine synthesis at pH 7.5. Subsequently, Mills and Alexander (1976) concluded that although microorganisms might carry out enzymatic nitrosations in some soils and waters, the NDMA could be formed nonenzymatically even at neutral pH's, and that, although pH was important, organic matter was perhaps more important. It was thought that one contribution of the microorganisms might be their influence on the organic matter. All of their systems were amended with high levels of amines and nitrite or nitrate.

Nitrosations in soils have been shown to occur with dimethylamine and trimethylamine when high levels of the amine and nitrite or nitrate are added. Pancholy (1976) observed the formation of NDMA after addition of both nitrite and dimethylamine to soil; the concentration increased for 12-15 days, then decreased to near zero by 30 days. High levels of inorganic nitrogen retarded the decomposition of the nitrosamine. Pancholy also analyzed polluted and fertilized soils for nitrosamines, nitrite, and nitrate; only the latter was observed. If secondary amines (10 ppm) were incubated with these soils, 0.1-0.5 ppm of nitrosamines were formed. Addition of glucose increased the amounts of nitrosamines formed, and little nitrosamine formation occurred in autoclaved soils. These results are in good accord with those of Verstraete and Alexander (1971), where nitrosamine formation seemed to be favored by anaerobic conditions. Although NDMA was produced from both tri- and dimethylamine, no nitrosamine was detected from several other organic compounds containing dimethylamino groups, including the herbicide, monuron. Nearly all of the reported formations of nitrosamines in soils have appeared to require relatively large amounts of added nitrite.

The herbicides atrazine-^{14}C (Kearney et al., 1977) and butralin-^{14}C (Oliver and Knotson, 1978) were found to form nitrosamines in soil, but only when high levels of sodium nitrite were added; no nitrosations of these herbicides were observed when ammonium nitrate was substituted for sodium nitrite. Interestingly, the nitrosoatrazine formed rapidly, but then rapidly disappeared; nitrosobutralin also formed rapidly, but was still detectable after 6 months. Tate and Alexander (1974) reported the formation in soil of dimethylamine and diethylamine from dimethyldithiocarbamate and diethyldithiocarbamate, produced traces of a nitrosamine in soil in the presence of nitrite. The fungicide mylone (tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione) did not produce a nitroso derivative under similar conditions.

Tate and Alexander (1974) were unable to detect by GLC any nitrosamines in soil treated with nitrite and glyphosphate at elevated concentrations, but Khan and Young (1977) detected nitrosoglyphosate in several soils using a method for measuring non-volatile nitrosamines. Subsequent kinetic studies (Young and Khan, 1978) showed that glyphosate nitrosation to nitrosoglyphosate was third order, with an activitation energy of 9.5 k cal/mole. Although the herbicide glyphosate [N-(phosphonomethyl) glycine] could theoretically lose the phosphoro group to yield sarcosine (which could then form a nitroso compound), neither sarcosine nor nitrososarcosine was found in soil incubated with nitrite (Khan and Young, 1977).

No N-nitrosocarbamates could be detected in three soils receiving sodium nitrite (at 100 and 1000 ppm) and 20 ppm of three insecticides, carbaryl, carbofuran or propoxur (Miyamoto, 1978). The limit of detection was 0.2 ppm.

Fine et al. (1976) analyzed for NDPA, a contaminant of trifluralin, in the air and irrigation water from tomato fields in the Sacramento Valley, California, before, during, and after application of the herbicide trifluralin. No nitrosamine was found.

Fine and Rounbehler (1976) released preliminary data indicating the possible presence of several nitrosamines in New Orleans area drinking water. N-nitrosoatrazine was suggested as the probable identity of one of the nitroso compounds. Newby and Tweedy (1976) found no N-nitrosoatrazine in water samples from a variety of locations in the Mississippi River from Iowa to Mississippi.

3.4.2 Formation and Uptake in Plants

Information is limited on nitrosamines in plants. Dressel (1976a) demonstrated uptake of NDMA and NDEA added to soil by wheat and barley. Dressel (1976b) also examined barley, wheat, spinach, lettuce, carrots, and tomatoes for nitrosamines from soils treated with 0 to 123 kg/ha N for NDMA and NDEA but found none. Wheat was also examined for nitrosamines by Sander et al. (1975) who treated fields with heavy doses of nitrogen fertilizers and either of three secondary amines (dimethylamine, N-methylaniline, N-methyl-N-benzylamine) but again no nitrosamines were detected. The same workers found that several nitrosamines could be taken up from water by cress, but that plant levels decreased rapidly when the nitrosamine-containing water was replaced by clean water (Sander et al., 1975). They, therefore, concluded that nitrosamines did not tend to accumulate in green plant material. Dean-Raymond and Alexander (1976) found uptake of NDMA into lettuce and spinach and also showed that the same compound was readily leached from soil by water.

Field studies on uptake of NDPA and N-nitrosopendimethalin by soybeans were conducted by Kearney et al. (1979). Concentrations of 0, 0.1, 1, 10 and 100 ppb in soils resulted in no measurable residues of either nitrosamine in soybean seeds after 110 days. Residues were measured by ^{14}C and TEA analyses.

One report describes the detection of nitrosodiethanolamine in cured tobaccos that had been treated in the field with the growth regulator MH-30 (maleic hydrazide formulated as its diethanolamine salt). Tobacco on which the growth regulator had not been used contained none of the nitrosamine. Schmeltz et al., (1977) concluded that the nitrosation had occurred in the plant.

3.4.3 Formation in Animals

There has been much literature published on the formation of nitrosamines in animals, and a complete review of it is beyond the scope of this section. Some general principles and specific studies on pesticides are reviewed here. Formation of nitroso derivatives in mammalian organisms, primarily in the stomach, is presumed to occur from various amino compounds, especially from secondary amines of low or moderate basicity, in the presence of nitrite. Nitrosamine formation has been demonstrated indirectly through induction of various tumors by concomitant administration of the amines with nitrite to experimental animals. N-methylaniline, N-methylbenzylamine, morpholine, and piperazine are examples (Sander et al., 1975).

The concentration of amines and nitrite used in animal experiments are generally unrealistically high. For example, tumors were produced in dietary studies where 2500 ppm N-methylbenzylamine and 800 ppm nitrite were fed. When the nitrite concentration was reduced to 600 ppm or below no tumors were found, demonstrating clearly the influence of nitrite concentrations. Using ethylurea and nitrite, the lowest concentrations producing tumors were 500 ppm in feed and 500 ppm in drinking water, respectively (Sanders et al., 1975).

Rounbehler et al. (1977), demonstrated the formation of NDMA in mice after gavage administration of 50 ng each of sodium nitrite and dimethylamine hydrochloride. Their procedure involinvolved blending the entire animal in liquid nitrogen, vacuum distillation of the resulting powder with mineral oil, and detecting NDMA by GC-TEA. The same group (Fine, et al., 1977) detected NDMA and NDEA in the blood of human volunteers after a meal that included bacon, spinach, tomatoes, and beer.

There are many factors affecting formation of nitroso derivatives in mammals. Thiocyanate in saliva and gastric juice enhances nitrosation reactions, whereas ascorbic acid reduces the yield of nitroso products not only in vitro, but also in vivo (Sanders et al., 1975, Douglass et al., 1978). Several phenolic compounds (gallic acid, tannic acid, α-tocopherol), as well as sulfur compounds, like cysteine, glutathione and methionine, are known to inhibit nitrosation (Douglass et al., 1978). Because of these factors, together with either rapid resorption from the stomach or rapid metabolism, accurate determination of the nitroso derivatives produced in vivo is difficult (Sander et al., 1975). Transnitrosation reactions may also contribute to a certain extent to the turnover of nitroso derivatives formed.

Only trace amounts of nitrosamines were detected in vivo in the stomach of rats or guinea pigs after concurrent administration of insecticidal carbamate compounds and nitrite (Miyamato and Hosokawa, 1977); when 16.2 mg (100 μ mole equivalent) ring-labeled m-cresyl N-methylcarbamate (tsumacide) was orally administered to male Sprague-Dawley rats concurrently with a 4-fold excess of sodium nitrite, less than 0.1% of the nitrosocarbamate was detected after 15 and 60 min. On the other hand, 60 min after oral administration of 4 mg/rat of the radioactive nitroso tsumacide, 55% of the radiocarbon and 41% of the nitroso tsumacide were found in the stomach, whereas no intact nitroso tsumacide was detected in intestines or in blood at 15-min postreatment. In starved rats and the stomach ligated at the pylorus (gastric juice pH, 2.0 to 2.2), the administered 16.2 mg/rat of radioactive tsumacide together with nitrite yielded 0.35% of the nitroso carbamate. At pH 1.2 to 1.4, in the gastric juice of guinea pigs

16.2, 162 or 0.16 mg/animal of radioactive tsumacide with four-fold excess of nitrite produced maximally 1.46, 0.41 or 0.11%, respectively, of nitroso tusmacide during 60 min (Miyamoto and Hosokawa, 1977). Marco et al., (1978) reported that rats fed atrazine with nitrite produced no detectable nitrosoatrazine (detection limit of 1 to 10 ppb) in stomach contents, stomach wall or excreta. Goats fed atrazine with nitrite produced no detectable nitrosoatrazine in liver, processed muscle, milk and excreta.

Maximal tolerated doses (10 to 100 mg/kg) of benzthiazuron, carbaryl, carbofuran, dimethoate, ethiofencarb, formetanate, linuron, maneb, methabenzthiazuron, propham or propoxur given orally to mice together with nitrite developed no increased micronuclei in bone marrow erythrocytes. In contrast, ETU produced a significant increase of micronucleated polychromatic erythrocytes under the similar conditions (Seiler 1977). This finding might exclude the possible formation of a measurable amount of the respective nitroso pesticide in living mice, although the sensitivity of the detection method is unclear.

Han (1973) added 1 ppm of ^{14}C-methomyl to macerates of commercially purchased cured meats (ham and hot dog) containing 16 to 20 ppm of residual sodium nitrite, and incubated the mixture under simulated stomach condition (pH 2) at 37°C. No nitrosomethomyl (less than 1 ppb) was found after 1 and 3 hr of incubation.

Several pesticides can be metabolized to dialkylamines when combined with high nitrite concentrations and acidic conditions and can form nitrosamine. Sen et al. (1974) fed the fungicides thiram, ziram and ferbam to guinea pigs with an excess of nitrite, and obtained very low levels of NDMA in the stomach. In a similar study, Eisenbrand et al. (1974) administered ziram with a 40-fold molar excess of nitrite to rats, and obtained an average yield of NDMA of 0.9%.

4. DEGRADATION AND METABOLISM

4.1. Degradation of Nitrosamines in Water and Air

There is limited information on the stability of nitrosamines associated with pesticides in natural waters. Preussman (1975) showed Cu^{2+}, OH^- ions enhanced the decomposition of ethylnitrosourea in aqueous solution. The Ni^{2+} ion showed similar but less pronounced effects. The decomposition rate of N-methyl-N'-nitro-N-nitrosoguanidine, a compound known to be relatively stable in aqueous solution, is strongly enhanced by the addition of Cu^{2+}. However, the stability of N-methyl-N-nitrosourethane is not influenced by heavy metal ions. In contrast, nitrosodialkylamines seem stable in water. NDMA, NDEA, and NDPA were not degraded in lake water during 3.5-month period (Tate and Alexander 1975).

Studies on N-nitrosoatrazine (NNA) in water revealed that the compound was stable towards hydrolysis over 3 weeks in water buffered at pH 5.5 and pH 8.0 or in river water at pH 7.1 (Wolfe et al., 1976). The NNA was rapidly decomposed by sunlight, however, yielding desethylatrazine and atrazine. Based on spectral and quantum yield data, the calculated half-life for photodecomposition of NNA in surface water was less than 10 min throughout the year in the United States. The authors concluded that sunlight photolysis will likely prevent any buildup of NNA in the aquatic environment. In a fish study with the combination of ^{14}C-atrazine and sodium nitrite in the water, no NNA was observed to be generated by the fish and none was found in the water. However, concentration of the water on a rotary evaporator at 40-50° did generate NNA, whereas lyophylization did not. Thus, sample preparation could lead to incorrect analytical results and misleading metabolic interpretations in the case of nitrosamines due to artifactual generation of the compounds (Marco et al., 1976).

There are only a few known studies concerning the photocomposition of nitrosamines in the vapor state. The aliphatic N-nitrosamines generally are rather volatile and may be expected to enter the atmosphere readily. NDMA vapor has been shown to be unstable to ultraviolet light, and so the simulated atmospheric degradation of NDPA was examined in a laboratory photoreactor. This nitrosamine was transformed with a half-life of less than 7 days into N,N-dipropylnitramine, which itself degraded to several products including N-dipropylpropionamide (Crosby, et al., 1978).

4.2. Fate of Nitrosamines in Soil

There is conflicting literature on the stability of nitrosodialkylamines in soils and on the role of soil microorganisms in their degradation. Ayanaba et al. (1973) reported that over 90% of the NDMA formed from dimethylamine disappeared in about 9 days after reaching a peak concentration of about 1.2 ppm. In contrast, Tate and Alexander (1975) found that NDMA was not degraded in flooded soil or in microbial enrichments from bog sediments. Likewise, NDEA and NDPA were not metabolized by enrichment cultures from soil or sewage. It was proposed that nitrosamines may persist in environmental samples because of the resistance of the

nitrogen-nitrogen bond to microbial attack. Additional studies by Tate and Alexander (1975) indicated that for NDMA, NDEA and NDPA, a lag of nearly 30 days occurred before their slow disappearance from soil; they disappeared slowly from sewage, but a minimum of 50% remained after 14 days. These results suggested a microbial involvement in the slow decomposition of the nitrosamines.

In degradation studies of NDPA-^{14}C in aerobic soils conducted in biometer flasks, ^{14}C losses by volatilization initially completed with losses of $^{14}CO_2$-production (Oliver et al., 1979). After a few days, however, $^{14}CO_2$ accounted for all of the additional ^{14}C trapped. Sterilization of the soil by either steam or ethylene oxide inhibited $^{14}CO_2$ production, but extended the time period over which NDPA volatilization was observed. Both NDMA and NDEA were degraded at rates similar to that of NDPA. The rate of $^{14}CO_2$ production was the same whether the NDPA was labeled at carbon 1, 2, or 3, and the half-life was estimated to be about 3 weeks. They concluded that the degradation was at least partly microbiological, and that once degradation began, the reaction probably proceeded rapidly all the way to CO_2. Saunders et al. (1979) studied the dissipation of NDPA from soil under both laboratory and field conditions. The results of their laboratory studies were consistent with those of Oliver and coworkers just discussed. In the field study, more than 90% of the NDPA incorporated in the top 10 cm of soil in 30-cm cylinder had dissipated within 3 weeks; modes of loss presumably included volatilization, degradation, and leaching. With heavy rainfall, some NDPA leached into the 10-20 cm section, but further leaching was not observed. The NDPA also dissipated from anaerobic soil, but somewhat less rapidly than from aerobic soil (Saunders et al., 1979).

The low molecular weight nitrosamines (NDMA, NDPA) volatilized very rapidly after surface application to moist soil (Oliver, in press); nearly 80% of the NDMA was lost in a few hours, and volatilization of NDPA was only slightly slower. As expected, incorporation of the nitrosamine into the soil (as would be the case if it were associated with a dinitroaniline herbicide) reduced both the rate and extent of volatilization. Volatilization of incorporated NDPA and NDEA differed somewhat from that of certain pesticides in that volatilization of NDPA and NDEA essentially ceased within 2-4 days in spite of the fact that additional nitrosamine remained in the soil. Nitrosopendimethalin was very nonvolatile, even after surface application.

The stability of synthetic nitrosopropoxur, nitrosocarbofuran, and nitrosocarbaryl in three soils in absence of light and after irradiation by natural sunlight has been examined by Miyamoto (1977). The initial disappearance of N-nitrosocarbamates from soil exposed to sunlight was extremely rapid, the half-life being 5 to 25 min, followed by a gradual decrease. Regardless of soil properties, the rate of disappearance was nitrosoporpoxur < nitrosocarbofuran < nitrosocarbaryl. In the dark, these nitrosocarbamates were more stable and after 12 hr about 80% of the added compound had disappeared.

Kearney et al. (1977), found that only 12% of ^{14}C- N-nitrosoatrazine could be recovered from aerobic Matapeake loam after 1 month, and after 3 and 4 months, the recovery was less than 1%. Denitrosation degrading to atrazine was a major pathway. In contrast to NDPA and nitrosoatrazine, the N-nitroso derivatives of two secondary amine dinitroaniline herbicides, butralin (Oliver and Kontson, 1978) and pendimethalin (Oliver et al., 1979) were found to be relatively stable in aerobic soil, and significant portions could be recovered after 6 months. A streptomyces culture isolated from an aerobic soil was found to metabolize N-nitrosopendimethalin (Lusby et al., 1978). Reduction of a nitro group and hydroxylation of a ring methyl seemed to be the major reactions; in contrast to nitrosotrazine, nitrosopendimethalin showed little tendency to denitrosate. Nitrosopendimethalin was rapidly degraded in flooded anaerobic soil (Oliver and Smith, 1979; Oliver et al., 1979); in this case, reduction of a nitro group was the only reaction identified and the reduction product was relatively stable to the anaerobic conditions.

4.3. Degradation of Nitrosamines in Plants.

Only a few reports on nitroso pesticides are known. Marco et al. (1976) showed that corn, grown to maturity in the greenhouse with a preemergence treatment of N-nitrosoatrazine (NNA) or N-nitrosohydroxyatrazine (NNHA) in soil treated with fertilizer containing nitrate and nitrite, did not contain either NNA or NNHA in the stalks or grain. Soybeans grown to maturity in soil treated with ^{14}C-NDPA or ^{14}C-nitrosopendimethalin showed no radioactive uptake of either compound in the mature beans (Kearney et al., 1978).

4.4 Metabolism of Nitrosamines in Animals

The metabolism of nitrosamines in mammals has been studied extensively during the last 20 years. The primary objective of most of the work has been the elucidation of the mechanism of the carcinogenicity of this class of compounds. The mode of action of nitrosamines is a topic of considerable importance but the present review will be concerned more with the pathways for their transformation by living organisms than with their effects on the organisms.

Most published work relates to NDMA and to lower molecular weight nitrosodialkylamines. A substantial amount of work has also been reported on cyclic nitrosamines.

4.4.1 Nitrosodimethylamine (NDMA). The metabolism, distribution in the body, and excretion of NDMA was studied by Magee (1956) in rats, rabbits, and mice using a polarographic method of analysis. Following the administration, the concentrations of the compound in most organs were similar. Nitrosamines show a remarkable organ specificity in their carcinogenicity and this specificity could not be explained by the preferential distribution of the compound in the body. On the basis of this and subsequent work, it is now firmly believed that the actual carcinogen is a metabolite of the original nitrosamine. Magee also showed that the liver was the main site for the metabolism of NDMA, in line with its toxic effects, and this has been amply demonstrated by later work (Dutton and Heath, 1956. Magee, 1972, Montesano and Magee, 1974). In the same work it was also shown that the rate of metabolism was rapid and only 34% of the NDMA could be recovered from a rat 8 hr after oral dosing. Only 1.7% of the NDMA was recovered from the urine within 24 hr and none in the feces.

Subsequent work (Dutton and Heath, 1956, Heath and Dutton, 1958) using ^{14}C-NDMA in the mouse and rat showed that 44-66% of the ^{14}C was eliminated as $^{14}CO_2$ within 6 hours with 6% in the urine of both species but with only traces in the feces of rats and none in mice. The results of these initial studies have been confirmed and extended *in vivo* and *in vitro*.

The initial step of the metabolism is considered to involve an enzyme-catalyzed demethylation via an unstable α-hydroxy compound (Druckrey *et al.*, 1967) that decomposes releasing formaldehyde (Brouwers and Emmelot, 1960, Magee and Hultin, 1962). The postulated sequence is shown in Figure 2.

In vitro, most of the carbon atoms in NDMA can be accounted for as methanol and formaldehyde (Lake *et al.*, 1976), and it has also been shown (Cottrell *et al.*, 1977) that all three hydrogen atoms in the methyl group originated from the methyl group of NDMA. Snyder and Malone (1976) investigated the metabolism of ^{14}C-NDMA in rats. Since methyl groups from NDMA are metabolized to formate, it would not be surprising that lipids are radioactive. However, most of the radioactivity in lipid extracts from livers of rats injected with ^{14}C-NDMA is specifically located in 3-sn-phosphatidycholine. These results indicated the transfer of methyl groups to lipids via the lipid methylation pathway that converts phosphatidylethanolamine to phosphatidylcholine.

The fate of the nitrogen atoms was first studied by Heath and Dutton (1958). Traces of methylamine, hydroxylamine, and nitrite were detected in liver and/or urine. These ^{15}N-studies showed that much of the amino nitrogen was converted into ammonia and that both nitrogen atoms in NDMA became evenly distributed in the nitrogen constituents of the body. Some recent data (Cottrell *et al.*, 1977) indicated that the formation of $^{15}N_2$ *in vitro* accounts for less than 5% of the conversion of NDMA into methanol and formaldehyde. On the other hand, it has been shown (Roller *et al.*, 1975) that α-acetoxynitrosodimethylamine, a precursor of α-hydroxynitrosodimethylamine, yields nitrogen quantitatively. Cottrell and his coworkers consider that their results shed considerable doubt on the degradative mechanism of NDMA that is indicated in Figure 2.

Methylation reactions occur with proteins (Magee and Hultin, 1962) and with the nucleic acids of RNA and DNA (Magee and Farber, 1962, Craddock and Magee, 1963, and Swann and Magee, 1968) and the methyldiazonium ion or a carbonium ion derived from it have been considered to be the methylating species. At one time it was considered (Rose, 1958, Schoental, 1960, Heath, 1961) that diazomethane could be the alkylating species. However, it has been demonstrated (Lijinsky *et al.*, 1968) that diazomethane itself is not an intermediate in the methylation of guanine by NDMA in DNA or RNA in rat livers. The reactions with proteins *in vitro* by ^{14}C-NDMA (Magee and Hultin, 1962) include methylation at the 1 and 3-positions of histidine. Some ^{14}C-NDMA activity was also detected in the 3-carbon atom of serine, indicating that the carbon atoms from NDMA had entered the C_1 metabolic pool. The main alkylation reaction of nucleic acids is at the N-7 position of guanine in RNA and DNA (Magee and Farber, 1962), although several other reactions have been noted including the methylation at the 1- and 3- position of adenine, the 1-position of cytosine, and the O-6-position of guanine (Lawley *et al.*, 1968, Craddock, 1973).

The alkylations of proteins and nucleic acids account for only a small percentage of the alkyl groups of NDMA. However, the latter reaction is widely believed to be the critical event in the carcinogenicity of nitrosamines. The correlation of carcinogenicity and the predominant methylation, N-7 of guanine, is not considered to be adequate and the arguments have been reviewed by Magee *et al.* (1975) and by Lijinsky (1976). It is now postulated that the alkylation at other sites, like the O-6 position of guanine in nucleic acids, may be even more critical events (Loveless, 1969). In studying NDMA methylase activity and its inhibitors, Friedman *et al.* (1976) concluded that nitrosarcosin and nitrosodiethylamine suppressed the enzyme activity in rat liver. They also concluded that DNA alkylation by NDMA in lung and kidney may be mediated by different pathways than is RNA or protein alkylation in the liver.

Singer (1975) has reviewed the methylation of ribose as well as the esterification of internucleotide phosphodiesters. All are considered to interfere with the proper functioning of DNA and RNA.

Overall, it has been shown that NDMA is metabolized rapidly, mainly in the liver, and is demethylated to 1 carbon intermediates. Most of these are oxidized to carbon dioxide or used in the normal metabolism of the body. The metabolism of NDMA has been studied extensively and a considerable work is still in progress. Much of the work in vivo has involved high and toxic doses and it would be worthwhile to consider some work at lower dosages.

4.4.2 Nitrosodiethylamine (NDEA) Heath (1962) showed that ^{14}C-NDEA, like NDMA, was metabolized rapidly in vivo by rats with most of the ^{14}C[1-C] being converted to CO_2. A small percentage of the dose (i.p. injection) was excreted unchanged in the urine (based on polarographic analysis) and this percentage decreased from 11% in 24 hours to 0.5% as the dose decreased from 200 to 50 mg/kg. It has been shown in vitro (Phillips et al., 1975) that in rat liver preparations much of the ^{14}C[1-C] in NDEA can be accounted for as ethanol and acetaldehyde, which agrees with the results of complementary studies with NDMA.

Alkylation of liver RNA of rats has been demonstrated in vivo (Magee and Lee, 1964) and there was evidence that the 7-position of guanine was being ethylated but not methylated (Kruger, 1972). This could occur as a result of α-oxidation of one ethyl group followed by transethylation of the second. The 7-ethylation of guanine in nucleic acids in rat liver and other organs has been confirmed (Ross et al., 1971, Swann and Magee, 1971) and the ethylation was shown not to involve diazoethane.

Oxidation of NDEA is feasible at the β-carbon atoms and the products of such reactions have been identified by TLC in β-glucuronidase-treated urine of rats given oral doses of NDEA (Blattman and Preussmann, 1973). N-Nitroso-N-ethyl-N-(2-hydroxyethyl)amine has been shown to be carcinogenic in rats (Druckrey et al., 1967).

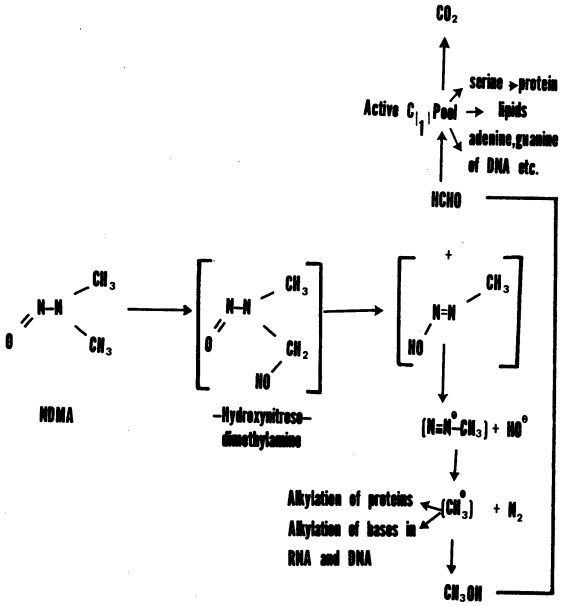


Fig. 2. Proposed metabolism of NDMA.

It has been shown (Schoental et al., 1974) that after the administration of NDEA to lactating rats (130 mg/kg by stomach tube), small amounts of unchanged NDEA (5-36 ppm) could be detected in the stomachs of suckling young within 6 hr after the beginning of suckling.

Overall, apparently NDEA metabolism is similar to that of NDMA with the additional occurrence of β-oxidation with the urinary elimination of oxidation products still containing the intact nitrosamine group.

4.4.3 Nitrosodipropylamine (NDPA). Kruger (1971, 1972) demonstrated that dosing rats with 1-[^{14}C]-NDPA led to the formation of 7-[^{14}C]-n-propylguanine and 7-[^{14}C]-methlylguanine in the RNA of rat liver, but that no [^{14}C]-7-methylguanine was detected when 2-[^{14}C]-NDPA was administered. This indicated that during metabolism the 1 and 2 carbon atoms split with the C-1 atom involved in the subsequent methylation. The splitting was considered (Kruger, 1972, Kruger, 1973, Althoff et al., 1973) to follow β-hydroxylation and the mechanism was postulated to resemble fatty acid metabolism and to apply to all nitrosodialkylamines with more than two carbon atoms in an alkyl group.

Products (III-VI) of metabolism by β and also γ oxidation have been identified (Blattmann and Preussman, 1973, Okada et al., 1975) in the β-glucuronidase-treated urine of rats given NDPA orally.

V11 Risk Assessment

The risk assessment section of this report follows closely the rational presented by Eli Lilly in their analyses of trifluralin. The following sections were prepared by J. R. Murphy and M. E. Amundson of the Lilly Research Laboratories.

Exposure Measurement

The demonstrated carcinogenicity in animals of certain nitrosoamines strongly recommended that information be obtained on possible human exposure to nitrosamines contained in pesticides. The obvious routes of exposure include: a) consumption of food or water that may contain residues of the compound, b) exposure of the agricultural worker during mixing and application of the pesticide, c) exposure of workers who enter treated areas after pesticide application to perform some task like retreatment, cultivation or harvest, d) exposure of the industrial worker in the production of the chemical, and e) exposure of the public from production waste stream contamination of waterways or solid waste disposal in landfills. Items d) and e) will not be discussed herein.

Reliable estimates of potential exposure can best be made by actual measurements under field conditions using proven sampling instrumentation and appropriate analytical techniques (Ross et al., 1978). Samples of treated crops should be obtained from fields or orchards treated with the chemical at the maximum rate and frequency of application. Consideration should also be given to possible animal tissue residues that may result from the consumption of forage crops by meat-producing animals. Samples of water from wells, ponds, irrigation canals, and waterways in or near the area of application should be obtained to assess potential contamination.

Several investigators (Jeiger, 1964; Wolfe et al., 1967; and Corner et al., 1975) have demonstrated measurable dermal and inhalation exposure of agricultural workers to pesticides during mixing and application operations. Direct exposure to the formulated pesticides through accidental spillage or contact with the spray mixture and to the spray mist is a possibility.

Exposure as a result of air, dust, and particulate contamination can be estimated by using portable sampling devices that have demonstrated a capability for measuring the compound in question, and that can be worn by the worker during the time of potential exposure. The extent of exposure to air and dust concentrations and to surfact contamination by the suspect chemical upon reentry into a treated area can be similarly assessed.

Equipment is available which will readily separate respirable-sized particles from air-borne dust samples. When the suspect chemical is present in the pesticide formulation at trace levels only, this equipment may not permit the collection of sufficient sample for analysis, and alternative methods of particulate collection, like vacuum cleaning devices, may be necessary to collect larger samples. It may also be necessary, in the case of trace contaminants, to use larger pumping devices capable of sampling larger volumes of air.

Dermal exposure can be assessed by appropriate analyses of special clothing, like shirts and gloves, worn by the field workers during the workday, or by using special collection or trapping devices worn by the workers.

Whenever samples are taken to measure environmental exposure, control samples and carefully designed recovery samples should be prepared in a manner that resembles as closely as possible the field conditions and location. This is especially important in the exposure assessment of labile chemicals, like nitrosamines. Recovery determinations from clothing exposed to wind and sunlight may lead to low or no recoveries due to volatility and photodecomposition of the suspect compounds.

The amount of exposure of agricultural workers who might enter a treated area after pesticide application may be assessed by using portable monitors to sample air, dust, and particulates, or by using stationary monitors that can be set up to take samples at various locations within the treated area. If contact is made with treated surfaces, like in harvesting fruit, dermal exposure should also be assessed. All samplings should coincide with actual entry intervals to properly assess potential exposure.

If dermal exposure to a suspect chemical is indicated, it may become necessary to obtain information on the rate and extent of absorption of the chemical to more fully evaluate the risk from such exposure. Models for these studies can be established using laboratory animals and radiolabeled compounds, although direct correlation of animal data with absorption in humans has not been firmly established.

Once the total human exposure to dietary, air, and dermal concentrations of the suspect chemical has been determined or estimated, the resultant risk from such exposure can be estimated.

Risk Assessment

The assessment of human cancer risk from exposure to a suspected carcinogen is a process of mathematically relating the measured human exposure, as described above, to observed carcinogenic effects of the substance. Before calculating the risk, carcinogenic effects of the substance in question must be quantified; and, generally, an extrapolation must be made from experimental and/or epidemiological data to measured or projected human exposure levels.

Two primary sources of information for quantifying the carcinogenicity of a substance are human epidemiological studies and studies using laboratory animals. Analysis of human epidemiological information avoids the problem of interspecies extrapolations, but it can be difficult to discern cause-and-effect relationships in the presence of inherent uncontrolled and unmeasured conditions. The detection of increased cancer incidence in a human population through epidemiological studies is most successful in cases where the cancer is of a rare form, where the target population is highly localized and specific, or where the information has been accumulated over a long period of time. No epidemiological information relating to nitrosamine exposure seems to be available.

Laboratory studies with animals, however, are usually conducted under carefully controlled conditions, with well-defined dose levels, and the carcinogenic effects of the compound under study can be directly observed. The difficulty with animal studies is relating or extrapolating the rather high exposure levels of the animals to much lower potential exposure levels in human populations.

Extrapolation from animal studies to human exposure generally involves predicting the response at dose levels considerably below the lowest dose producing an observable response, and the predicted response is, therefore, a function of the dose-response model used. Controversy is considerable in the scientific community concerning how extrapolation should be calculated. The present state of knowledge does not permit precise determination of what form the dose-response should have at low doses. Some approaches to the problem of low-dose extrapolation which have been proposed are discussed.

One of the most common mathematical forms used to represent a carcinogenic dose-response relationship is the log-probit model. This model is widely used in biological assay and involves the assumption of a linear relationship between the logarithm of the dose and the probit transform of the proportion of organisms that respond at each dose level. The method of estimating the associated parameters is referred to as probit analysis, as described by Finney (1964).

The first approach to the low-dose extrapolation problem was proposed by Mantel and Bryan (1961). The essentials of the method entail the development of conservative statistical upper limits on the proportion which could have responded at each dose level, and the downward extrapolation from the most conservative of these with a conservative log-probit slope. The authors suggested that a conservative slope could be taken as one probit per log unit. The method was later "improved" and extended by Mantel *et al.* (1974).

Cornfield (1977), in a critique of the Mantel-Bryan extrapolation technique as well as a discussion of the general use of "conservative" procedures, indicated that a conservative approach to risk assessment tends to distort any risk-benefit assessment by comparing

exaggerated risks with determinations of benefit. He proposed that it is appropriate to compare expected risks versus expected benefits, attaching to each the proper weights, rather than arbitrarily assigning great weight to the risk without regard to the possible benefit.

An approach to low-dose extrapolation based on kinetic considerations was also proposed by Cornfield (1977). The treatment closely resembled that of Gehring and Blau (1977). The unique feature of these kinetic models is that they allow for the possibility that threshold doses exist, i.e., doses below which there is no carcinogenic response. Their practical application requires that the values of associated kinetic rate constants be estimated by some method.

Another mathematical form, which has been found useful in radiation carcinogenesis, is the one-hit exponential model: $p = 1 - \exp(-k*dose)$, where P = probability of response. The model is derived from the premise that carcinogenesis arises as the result of genetic "hits", and follows from the assumption that the hits are governed by a Poisson process. The bases for the one-hit exponential model and the log-probit model are fundamentally different.

Altschuler (1976) described a Bayesian method of risk extrapolation based on the one-hit exponential model. He proposed dealing with the lack of data at lower doses by applying subjectively-assigned modifying factors covering degree of conservatism, transferability of animal results to humans, and the probability of a substance being both an animal and human risk is obtained. Altschuler's approach has not gained wide acceptance, partly due to its dependence on the one-hit exponential model, and partly due to its reliance on Bayesian techniques.

Another approach is one-hit linear extrapolation. The near-linearity of the one-hit exponential model at low doses is sometimes used as a basis for low-dose extrapolation in studies involving chemical carcinogenesis. Linear extrapolation is regarded by most authorities as the most conservative of all methods. It has been criticized primarily because it involves the implicit stipulation that the response is a linear function of dose for humans, independently of whether such a relationship is supported by available animal data.

The most general of all the solutions proposed to date is that of Hartley and Sielken (1977) designed to be applied in cases where time-to-tumor information is available. However, the solution used where only tumor incidence counts have been reported. The authors propose the use of a hazard function formed as the product of two polynomials with unknown coefficients, and develop a convex-programming computer algorithm to calculate approximate maximum likelihood estimates of the coefficients. Although the calculations are somewhat complex, low-dose extrapolation is reasonably straightforward once the unknown parameters have been determined.

A major shortcoming of all of the above methods is their attempt to deal with prediction of the response for dose levels where no data are available. Close agreement between the model and the data are considered essential with all of the methods, except the one-hit linear extrapolation. Selection of the extrapolation model to be used, then, must be based on other considerations. Altschuler's Bayesian approach is not acceptable to those who do not subscribe to the principles of subjective probability. Similarly, the kinetic models of Cornfield and Gehring-Blau are probably not sufficiently developed to permit immediate application. For cases where time-to-tumor information is available, the Hartley-Sielken approach is appropriate, since the Mantel-Bryan technique is not designed to handle such data. However, the estimation procedure associated with the Hartley-Sielken model is somewhat complicated and cannot be easily performed with a computer. For cases where only tumor incidence is known, it is questionable whether the results obtained from application of the Hartley-Sielken method warrant the extra effort and expense. In addition, the technique contains no explicit provision for adjustment between species. The Mantel-Bryan procedure has been modified by its authors to the point where its application without the computer is also impossible. Further, due to the fact that the Mantel-Bryan approach, like that of Hartley-Sielken, was designed for "safe dose" estimation, rather than simple risk estimation, conservatism of one degree or another is built into the analysis at several points.

In view of the fact that almost all of the laboratory animal studies with nitrosamines provide only tumor incidence data, and since such data generally seem to conform to a log-probit dose-response model, an approach to nitrosamine risk assessment may be to use a less conservative modification of the Mantel-Bryan procedure. Such an approach could be implemented by fitting a log-probit model to the animal data, making a species adjustment to humans, and then extrapolating downward to a selected incidence rate, i.e., 10%. Further downward extrapolation from that point could be accomplished using a conservative log-probit slope of one probit per log unit. This method was used to derive risk estimates based on exposure to NDPA, which was found to be a contaminant in the herbicide trifulralin, and has been published (Federal Register, 1977). An example of a risk assessment calculation is given in the Appendix.

Once a risk assessment has been completed and the potential hazard of a chemical to the exposed human population has been estimated, it becomes important to also consider the benefits of continued use of the chemical before any rational decision can be made as to the ultimate fate of the chemical or product in question.

APPENDIX

Sample Risk Assessment Calculation

The estimation of cancer risk to a human population due to exposure to a carcinogen, like nitrosamine, is a three-step process:

1. Identification of all potential sources and means of exposure and the determination of actual exposure levels.
2. Quantification of the carcinogenic effects of the compound from studies on laboratory animals.
3. Extrapolation of the effects observed in animals to man, and extrapolation from the dose-levels of actual experimentation to the dose-levels of projected exposure.

Assumptions

1. A herbicide formulation contained dimethyl-N-nitrosamine (DMNA). Exposure studies indicated that the exposure of the agricultural worker was limited to the preparation of the spray mixture and application of the herbicide. The inhalation exposure was calculated to be 1 µg/year and the dermal exposure, 5 µg/year.
2. The average working lifetime of an agricultural worker is 30 years, and the average lifespan is 70 years. The average daily lifetime inhalation exposure is 1 µg/year x 30 years ÷ 70 years x 365 days/year = 1.174×10^{-3} µg/day, and the average daily lifetime dermal exposure is 5 µg/year x 30 years - 70 years x 365 days/year = 5.87×10^{-3} µg/day.

Animal Dose-Response Relationships

Dietary Studies

The data from a NDMA dietary study (Terracini et al., 1967) seemed to conform to a log-probit model, and the slope and ED_{50} (dose estimated to produce tumors in 50% of animals tested) are:

RAT FEEDING STUDY WITH NDMA

Cited Dosage	Calculated Daily Dosage	Tumor Incidence		Probits Observed	Probits Calculated
ppm	mg/hg/day		(%)		
2	.1	1/27	(3.7)	3.21	2.767
5	.25	5/68	(7.35)	3.55	3.740
10	.5	2/5	(40)	4.75	4.475
20	1.0	15/23	(65)	5.39	5.211
50	2.5	10/12	(83.3)	5.97	6.183

$ED_{50} = 0.82$ Slope = 2.4432 Probits/$\log_{10}$ unit.

Inhalation Studies

None of the three reported studies (Druckery et al., 1964; Druckery et al., 1967; Moiseev and Benemanskii, 1975) provide reliable dose-response information. However, assuming that a log-probit model applies to these data, and assuming the slope is the same as for the dietary study, the calculations are:

INHALATION STUDIES WITH NDMA

Study[1]	Cited Dosage	Calc. Avg. Daily Dose (μg/kg/day)	Tumor Incidence	(%)	Calc. ED_{50} (μg/kg/day)
(i)	1x37 mg/kg	123	1/3	(33.3)	185
(ii)	2x2 mg/kg/wk	571	8/12	(66.7)	380
	2x4 mg/kg/wk	1143	6/6	(100)	-
(iii)	5 $\mu g/m^3$	7	**[2]	(15)	-
	219 $\mu g/m^3$	317	31/83	(26.3)[3]	579
(iii)	5 $\mu g/m^3$	3	**[2]	(30)	-
	219 $\mu g/m^3$	135	38/51	(63.6)[3]	97

[1] (1) Druckery et al. (1964) - single dose inhalation with rats
(ii) Druckery et al. (1967) - inhalation twice weekly with rats
(iii) Moiseev and Benemanskii (1975) - continuous inhalation with mice and rats

[2] ** Not statistically different from control

[3] Adjusted percentages calculated by Abbott's formula P' - (P-C)/(1-C).

Dermal Studies

None have been reported. Assume 30% absorption and similar effects as if ingested.

Extrapolation

Development of Human Dose-Responses

Assumed body weights for mouse, rat and human are 20, 300 and 70 kg, respectively. Using the body surface area rule, where W_A and W_B are body weights of species A and B and the slope and ED_{50} values are the same with respect to similar mode of exposure, the ED_{50} values are the same with respect to similar mode of exposure, the ED_{50} for humans is estimated by:

$$ED_{50}\ (B) = (W_A/W_B)^{1/3} \times ED_{50}\ (A), \text{ and}$$

for oral exposure is:

$$ED_{50}\ (\text{human}) = (0.16243)\ (0.82) = 0.1332 \text{ mg/kg/day},$$

or for a 70-kg human, 0.1332 x 70 = 9.3234 mg/day.

From the three inhalation studies evaluated, four estimates of the ED_{50} (human) can be made:

E_1 = (0.16243)(0.185) - 0.03 mg/kg/day
E_2 = (0.16243)(0.380) = 0.06173 mg/kg/day
E_3 = (0.06586)(0.579) = 0.03813 mg/kg/day
E_4 = (0.16243)(0.079) = 0.01576 mg/kg/day.

The geometric average is 0.03247 mg/kg/day, or for a 70-kg human, 0.03247 x 70 = 2.273 mg/day.

Based upon an assumed 30% absorption resulting from dermal contact with DMNA and the same toxicological effect as if the compound were ingested, the dermal exposure ED_{50} (human) would be 9.3234 mg/day, (the oral ED_{50}) - 0.30 = 31.08 mg/day.

Extrapolation to Low Exposure Dose Levels

The human exposure levels estimated to produce a 10% cancer incidence rate from inhalation and dermal exposure are:

$$2.273 \times 10^{(-1.28/2.4432)} = 0.6803 \text{ mg/day (inhalation)}$$

$$31.03 \times 10^{(-1.28/2.4432)} = 9.302 \text{ mg/day (dermal)}.$$

The measured exposure levels calculated earlier were 1.174×10^{-3} μg/day by dermal exposure. Using both linear extrapolation, and a log-probit extrapolation with an assumed slope of 1 probit/$\log_{10}$ unit, the risk can be stimated as follows:

Linear extrapolation:

$$\text{Inhalation risk} = (0.1)(1.174 \times 10^{-3})/680.3 = 1.726 \times 10^{-7}$$
$$\text{Dermal risk} = (0.1)(5.87 \times 10^{-3})/9302 = 6.31 \times 10^{-8}$$
$$\text{Total risk} = 2.36 \times 10^{-7} \text{ or } 1 \text{ in } 4.24 \text{ million}$$

Log probit extrapolation:

$$\text{Inhalation risk} = Z(\text{risk}) = -1.28 - \log(680.3/1.174 \times 10^{-3}) = -7.043, \text{ a risk of } < 1 \times 10^{-12}$$
$$\text{Dermal risk} = Z(\text{risk}) = 1.28 - \log(9302/5.87 \times 10^{-3}) = -7.48, \text{ a risk of } < 1 \times 10^{-11}$$
$$\text{Total risk} = < 1.1 \times 10^{-11}, \text{ or } < 1 \text{ in } 90 \text{ billion.}$$

Conclusions

1. Nitrosamines are associated with a few, chemically definable, predictable and related classes of pesticides and do not occur randomly in pesticide chemicals.

2. The concentration of nitrosamines in the formulated product can be effectively lowered by improved process and formulation techniques.

3. The nitrosodialkylamines associated with pesticides are unstable in environmental systems and are rapidly degraded in soil, water, and air.

4. Two synthetic nitrosated secondary amine herbicides are relatively stable in soils.

5. Many synthetic nitrosated pesticides are chemically unstable and exist only under laboratory conditions.

6. Current analytical methodology, including thermal energy analysis, offers reasonably sensitive and specific methods of quantitative analysis but must be confirmed.

7. Environmental formation of pesticidal nitrosamines is rare, but may occur in animal stomachs at low pH and high nitrite concentration.

8. Model equations exist, which coupled with necessary assumptions, allow the risks associated with nitrosamines in pesticides to be evaluated.

Recommendations

1. Reduce the nitrosamine concentration in pesticides and their precusors by whatever means possible.

2. Pesticides and their precursors should be screened routinely for nitrosamine content where the chemistry indicates.

3. A practical and reasonable limit should be established for the nitrosamine content in pesticides.

4. Independent analytical confirmation techniques must be used to verify any reported nitrosamine associated with pesticides.

5. Additional environmental and related studies are needed on some of the nitrosated pesticides because of limited available information.

6. The analyst should be cautioned about the possible artifactual formation of nitrosated compounds during sample preparation.

7. The risks associated with the nitrosamine-in-pesticide issue needs to be related to those assessed for other sources of environmental exposure to nitrosamines.

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