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HEALTH AND ENVIRONMENTAL EFFECTS DOCUMENT FOR 3-NITROANILINE

Prepared for

OFFICE OF SOLID WASTE AND
EMERGENCY RESPONSE

Prepared by

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PREFACE

Health and Environmental Effects Documents (HEEDs) are prepared for the Office of Solid Waste and Emergency Response (OSWER). This document series is intended to support listings under the Resource Conservation and Recovery Act (RCRA) as well as to provide health-related limits and goals for emergency and remedial actions under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained for Agency Program Office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched for in this document and the dates searched are included in "Appendix: Literature Searched." Literature search material is current up to 8 months previous to the final draft date listed on the front cover. Final draft document dates (front cover) reflect the date the document is sent to the Program Officer (OSWER).

Several quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include: Reference doses (RfDs) for chronic and subchronic exposures for both the inhalation and oral exposures. The subchronic or partial lifetime RfD, is an estimate of an exposure level which would not be expected to cause adverse effects when exposure occurs during a limited time interval i.e., for an interval which does not constitute a significant portion of the lifespan. This type of exposure estimate has not been extensively used, or rigorously defined as previous risk assessment efforts have focused primarily on lifetime exposure scenarios. Animal data used for subchronic estimates generally reflect exposure durations of 30-90 days. The general methodology for estimating subchronic RfDs is the same as traditionally employed for chronic estimates, except that subchronic data are utilized when available.

In the case of suspected carcinogens, a carcinogenic potency factor, or q_1^* (U.S. EPA, 1980), is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively. An RfD may also be derived for the noncarcinogenic health effects of compounds that are also carcinogenic.

Reportable quantities (RQs) based on both chronic toxicity nd carcinogenicity are derived. The RQ is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). These two RQs (chronic toxicity and carcinogenicity) represent two of six scores developed (the remaining four reflect ignitability, reactivity, aquatic toxicity, and acute mammalian toxicity). Chemical-specific RQs reflect the lowest of these six primary criteria. The methodology for chronic toxicity and cancer based RQs are defined in U.S. EPA, 1984 and 1986b, respectively.

EXECUTIVE SUMMARY

3-Nitroaniline is a synthetic chemical that has the appearance of yellow rhombic needles in its purified form. It is slightly soluble in water and readily soluble in organic solvents such as ethanol, ether and methanol. 3-Nitroaniline is used as an intermediate in the synthesis of dyestuffs and other organics. There are no data on producers or volume of production.

The available data indicate that the environmental fate of 3-nitroaniline is controlled largely by its water solubility, low vapor pressure, susceptibility to photodegradation, and by its affinity to bind with humic substances in soil and sediments. Thus, 3-nitroaniline is not expected to be a major atmospheric contaminant. It may be present in aqueous media, but probably only near point sources. Although it is not readily susceptible to biodegradation, its binding with humic substances in soils and sediments will probably limit its bioavailability. It has been reported in only a few monitoring studies, and exposure levels to the general public are probably minimal.

Information on the environmental toxicology of 3-nitroaniline is quite limited. In one study the acute LC_{50} to one species of freshwater fish was reported to be 51 mg/ℓ. There is no information on acute toxicity to saltwater species, or on chronic effects to either marine or freshwater organisms. The partition coefficient for 3-nitroaniline and experimentally derived and calculated bioconcentration factors indicate a low potential for bioaccumulation. Data for terrestrial vertebrates (birds and mice) indicate a relatively low acute toxicity of 3-nitroaniline, the oral LD_{50} values being >100 mg/kg.

Sufficient information is not available for a quantitative environmental risk assessment for 3-nitroaniline. Data are inadequate for deriving water quality criteria. There is only one specific data point for acute toxicity and no information on which to derive a Criterion Continuous Concentration. From a qualitative point of view, the available data on environmental persistence, toxicity, and bioaccumulation potential of 3-nitroaniline suggest that the chemical represents only a low level of environmental risk. Releases to the environment would be subject to physical and chemical degradation, and binding with soil and sediments is likely to limit its transport and bioavailability.

Data concerning health effects of 3-nitroaniline in mammalian systems are also limited. Pharmacokinetics data showed that 3-nitroaniline is readily absorbed through the lungs and from intact skin. Data regarding absorption from the gastrointestinal tract and tissue distribution were not found. One report suggested that 3-nitroaniline is excreted into urine as a conjugated or unconjugated form of the parent molecule (Wells et al., 1920-1921), and another report presented evidence that diazo-positive substances (indicators for the presence of nitro-amino derivatives) are excreted into urine of animals injected i.p. with 3-nitroaniline (Watanabe et al., 1976). The evidence also suggested that 3-nitroaniline, which is formed as a metabolite of 1,3-dinitrobenzene, is in turn metabolized to 4-amino-2-nitrophenol, 2-amino-4-nitrophenol, 1,3-diaminobenzene, and 2,4-diaminophenol (Rickert, 1987). Another study showed that 3-nitroaniline can be metabolized to N-oxidation products in a cell-free system (Corbett and Corbett, 1985).

With the exception of genotoxicity data, almost all toxicity data were limited to acute exposure. Lethality data for 3-nitroaniline show LD_{50}s

of 308 mg/kg for mice, 450 mg/kg for guinea pigs, and 535-900 mg/kg for rats (Vasilenko et al., 1974; Vernot et al., 1977; RTECS, 1989). Other data showed that dogs can be killed by a single i.p. injection of 70 mg/kg, guinea pigs by 212 mg/kg, cats by 218 mg/kg, and rabbits by 294-346 mg/kg. A single i.p. injection of 200 mg/kg causes no toxic effects in the rabbit (Wells et al., 1920-1921).

Clinical effects from acute exposure to 3-nitroaniline include dyspnea and convulsions before death, with postmortem signs of asphyxia (Wells et al., 1920-1921). 3-Nitroaniline is a methemoglobin and a sulfhemogloben former (DeBruin, 1976; Beard and Noe, 1981; Watanabe et al., 1976; Vasilenko et al., 1974; Vasilenko and Zveydai, 1974). Other toxic effects may include decreases in red and white blood cell counts, bone marrow changes indicative of anemia, pulmonary edema, and damage to the kidney, spleen, liver and heart (Wells et al., 1920-1921). Serum GOT and GPT activities in rats were not affected by i.p. doses of 100 μmol/kg (Watanabe et al., 1976).

No data were available regarding subchronic or chronic toxicity, carcinogenicity, developmental toxicity or reproductive toxicity of 3-nitroaniline.

Genotoxicity data showed that 3-nitroaniline is mutagenic in Salmonella typhimurium under various conditions. It has produced positive results in strains capable of detecting both base-pair substitutions (TA100 and TA1535) and frameshift mutations (TA98, TA1537 and TA1538). In general, concentrations $\geq$500 μg/plate are required to induce mutations in the absence of S9 (Chiu et al., 1978; Shahin, 1985; Shimizu and Yano, 1986). Lower concentrations (30-250 μg/plate) of 3-nitroaniline can induce mutations in the presence of S9 (Garner and Nutman, 1977; Thompson et al., 1983). 3-Nitroaniline preincubated with FMN in the presence of hamster or rat liver S9 can

induce mutations at concentrations as low as 6.9-27.6 µg/plate (Dellarco and Prival 1989). This study indicates that nitroreduction may indeed be a factor in the activation of 3-nitroaniline. Other genotoxicity tests showed that 3-nitroaniline induced a weak positive response in the Kada rec assay (Shimizu and Yano, 1986) and a negative response in the test for unscheduled DNA synthesis using rat hepatocytes (Thompson et al., 1983).

Data were not available for evaluating the toxicity of 3-nitroaniline based on carcinogenicity, subchronic exposure, or chronic exposure. Subchronic and chronic RfDs (inhalation and oral) and cancer and chronic RQs could not be calculated.

3-Nitroaniline is placed in weight-of-evidence group D, not classifiable as to human carcinogenicity. Qualitative evaluations of the potential carcinogenicity of compounds can sometimes be based on evidence for structural analogues or metabolites. Several possible metabolites of 3-nitroaniline were identified. 4-Amino-2-nitrophenol and 2-amino-4-nitrophenol were carcinogenic in male rats, but not in male and female mice. 4-Amino-2-nitrophenol was possibly carcinogenic in female rats and 2-amino-4-nitrophenol was not carcinogenic in female rats. Data were inadequate for evaluating the carcinogenicity of 1,3-diaminobenzene. All three compounds were mutagenic in Salmonella in the presence or absence of S9. Data regarding these compounds were not sufficient for altering the evaluation of 3-nitroaniline, because definitive evidence that these compounds are metabolites of 3-nitroaniline was not available and evidence for potential carcinogenicity was only "limited," that is, positive in only one sex of one species.

Literature on possible metabolites was also examined for evidence of developmental or reproductive toxicity. 1,3-Diaminobenzene was fetotoxic and demonstrated significant developmental toxicity when female rats were given 90 mg/kg bw/day during days 6-15 of gestation (Hruby et al., 1981).

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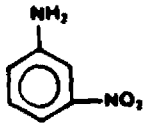
LIST OF ABBREVIATIONS

BCF	Bioconcentration factor
bw	Body weight
EC_{50}	Median effective concentration
FMN	Flavin mononucleotide
GOT	Glutamic oxaloacetic transaminase
GPT	Glutamic pyruvic transaminase
i.p.	Intraperitoneal
K_d	Soil adsorption coefficient
K_{oc}	Soil organic carbon/water partition coefficient
K_{om}	Soil organic matter/water partition coefficient
K_{ow}	Octanol/water partition coefficient
LC_{50}	Concentration lethal to 50% of recipients
LD_{50}	Dose lethal to 50% of recipients
M	Molar concentration
om	Organic matter
P_o	Saturation vapor pressure
RfD	Reference dose
RQ	Reportable quantity
S.E.M.	Standard error of the mean
$t_{1/2}$	Half-life
TL_m	Median tolerance limit

1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

Synonyms for 3-nitroaniline are as follows: benzenamine, 3-nitro (9CI); 3-nitro- aniline; 3-nitrobenzenamine; 1-amino-3-nitrobenzene; m-nitrophenylamine; Amarthol fast orange R base; and C.I. azoic diazo component 7. Its Colour Index number is C.I. 37030 (CHEMLINE computer printout, 1989). The struc- tural formula, CAS number, empirical formula, and molecular weight are as follows:



CAS No.: 99-09-2

Empirical formula: $C_6H_6N_2O_2$

Molecular weight: 138.14

1.2. PHYSICAL AND CHEMICAL PROPERTIES

3-nitroaniline is a solid that has the appearance of yellow rhombic needles in its purified form (Beard and Noe, 1981; Windholz et al., 1983; Weast et al., 1988). It has a burning sweet taste (Beard and Noe, 1981). It is soluble in aqueous solvents (1 g in 880 mℓ of water), ethanol (1 g in 20 mℓ), ether (1 g in 18 mℓ), and methanol (1 g in 11.5 mℓ) (Windholz et al., 1983). Other chemical and physical properties of 3-nitro- aniline are presented below (Windholz et al., 1983; Dean, 1987; Weast et al., 1988):

Melting point:	114°C
Boiling point:	305.7°C
Density:	1.43 (20/4°C); 1.1747 (160/4°C); 0.9011 (25/4°C)
Vapor pressure:	1 mm at 119.3°C

Log K_{ow}	1.37
pK_a	2.46
Conversion factor:	1 mg/m³ = 0.17 ppm at 20°C

1.3. PRODUCTION DATA

No producers or production data for 3-nitroaniline were found in the sources available; 167,000 pounds of 3-nitroaniline was imported in 1976 (USITC, 1976). Aceto Chemical Co. Inc., Flushing, NY stopped importing 3-nitroaniline after April 1980; its inventory as of March 1983 was sold off at a rate of 10,000 lb/year (Koeppe, 1983).

1.4. USE DATA

3-Nitroaniline is used primarily as an intermediate for the synthesis of dyestuffs and other organics (Beard and Noe, 1981; Windholz et al., 1983).

1.5. SUMMARY

3-Nitroaniline has the appearance of yellow rhombic needles in its purified form. It is slightly soluble in water and readily soluble in organic solvents, such as ethanol, ether, and methanol. 3-Nitroaniline is used as an intermediate in the synthesis of dyestuffs and other organics. No data on producers or volume of production were found.

2. ENVIRONMENTAL FATE AND TRANSPORT

2.1. AIR

No comprehensive study on the fate and transport of nitroanilines in the atmosphere was found in the literature. However, some projections have been made based on chemical structure and fate of these compounds in aquatic media (U.S. EPA, 1985).

2.1.1. Physical and Chemical Processes. Mononitroanilines are likely to undergo oxidation reactions in the atmosphere. The amino group may be especially susceptible to oxidation. Oxidation may occur through reactions with hydroxyl radicals or molecular oxygen (U.S. EPA, 1985). The half-life for the reaction of 3-nitroaniline with hydroxyl radicals in a typical ambient atmosphere was calculated by SRC (1989) to be about 14 hours based on an estimated reaction rate constant of 1.7×10^{-11} cm^3/molecule-sec at 25°C (GEMS, 1987). Direct photodegradation of 3-nitroaniline is also likely to occur, but kinetic data are not available to calculate photolytic rates in natural environments (U.S. EPA, 1985).

2.1.2. Transport. Organics present in the atmosphere can exist in the vapor phase or be adsorbed onto particulate matter. From theoretical considerations, the partitioning of these compounds between the vapor and aerosol phases depends on the saturation vapor pressure (P_0) of the compound and the size, surface area, and organic content of the particles (Junge, 1977). Generally, adsorption onto particulate matter occurs with compounds having P_0 values $<10^{-4}$ mm Hg, and compounds with P_0 values $\leq 10^{-8}$ mm Hg should occur almost entirely in the particulate phase (Eisenreich et al., 1981). The vapor pressure of 3-nitroaniline has been reported to be 0.000036 mm Hg; therefore, only limited adsorption onto atmospheric particles and dry deposition will occur (SRC, 1989). In contrast,

because of the high water solubility of nitroanilines, transport of 3-nitroaniline from the atmosphere to surface waters and soil through wet deposition is expected to be significant (U.S. EPA, 1985).

2.2. WATER

2.2.1. Physical and Chemical Processes. The water solubility of 3-nitroaniline has been reported to be 910 ppm at 25°C (Seidell, 1941), indicating that aqueous environmental media would be subject to contamination by this compound. There is no information available on the reaction of 3-nitroaniline with free radicals or singlet oxygen in aqueous media. Laboratory studies with 2-nitroaniline suggest that, in natural aquatic environments, hydrolysis would not be a significant reaction pathway (U.S. EPA, 1985). A similar conclusion can probably be made for 3-nitroaniline.

Ultraviolet absorption maxima for 3-nitroaniline have been reported to be 236, 275 (shoulder) and 374 nm (Weast and Astle, 1979). Consequently, in surface waters, 3-nitroaniline would be susceptible to direct photolysis and photooxidation (SRC, 1989). Laboratory studies have demonstrated that 2- and 4-nitroaniline undergo photolysis, and 3-nitroaniline can be expected to undergo the same fate. From monitoring data, Zoetman et al. (1980) estimated that the half-life of 3-nitroaniline in the Rhine River was 1.0 days.

2.2.2. Biodegradation. There are conflicting data on the susceptibility of 3-nitroaniline to microbial biodegradation in aqueous media. Malaney (1960) reported some oxidation of all three isomers of nitroaniline by aniline-acclimated activated sludge (as measured in a standard Warburg respirometer). In tests conducted with an electrolytic respirometer, Urano and Kato (1986a,b) found that a 100 mg/ℓ concentration of 3-nitro- aniline was not biodegraded by unacclimatized activated sludge (30 mg/ℓ) after 240 hours at 20°C. Similarly, Pitter (1976) and Kitano (1978)

reported no degradation of this compound, or of 2- and 4-nitroaniline, when incubated in the presence of adapted activated sludge. Partial degradation of 3-nitroaniline was reported by Chambers et al. (1963) in tests using phenol-adapted bacteria and by Young and Affleck (1974) in tests using a 4-nitroaniline-adapted sewage inoculum. Park et al. (1988) reported that 3-nitroaniline was one of a number of aromatic amines that could be used as a sole source of carbon by Pseudomonas putida cultures isolated from activated sludge. Paris and Wolfe (1987) reported that bacterial isolates obtained from river water were also capable of degrading 3-nitroaniline. For a bacterial concentration of 10^{13} organisms/ℓ, the disappearance rate constant for the reaction was $1.2 \pm 0.8 \times 10^{-15}$ ℓ (organism)$^{-1}$ hour^{-1}, and the calculated $t_{1/2}$ was 58.0 hours. For field samples obtained from three localities and containing estimated bacterial levels of only 10^{8} organisms per liter, the average $t_{1/2}$ was reported to be 1.7 years. The data indicated that the primary transformation pathway involved the oxidative deamination of the nitroaniline. Other studies on 2- and 4-nitroaniline have produced conflicting results, some indicating a small level of biodegradation and others none at all. The pathway of biodegradation of 4-nitroaniline by Pseudomonas and Bacillus was reported to occur through the formation of 4-phenylenediamine and 4-aminophenol intermediate products.

2.2.3. Transport. Based on the water solubility of 910 ppm at 25°C, and an estimated vapor pressure of 0.0000362 mm Hg at 25° (Ferro and Piancente, 1985), the Henry's Law constant for 3-nitroaniline was estimated by SRC (1989) to be 7.2×10^{-9}. This low value indicates that volatilization of 3-nitroaniline from aqueous media would be minimal.

When released in natural waters, 3-nitroaniline is likely to undergo covalent chemical bonding with humic materials in the water column and sediments (SRC, 1989). Information on the kinetics of this reaction is not available.

2.3. SOIL

2.3.1. Physical and Chemical Processes. On soil surfaces and when exposed to sunlight, 3-nitroaniline is expected to be susceptible to photodegradation (SRC, 1989). Data on the kinetics and degradation products of the reaction, however, are not available.

There is evidence that aromatic amines such as 3-nitroaniline bind to humic substances (SRC, 1989). Laboratory studies indicate that the reaction involves a chemical covalent bonding that is achieved through an initial rapid reversible step involving the formation of imine linkages with humate carbonyls, followed by a slower less reversible reaction possibly involving 1,4- addition to quinone rings and then tautomerication and oxidation to give an amino-substituted quinone (Parris, 1980). After the second step is reached, leaching of 3-nitroaniline from the soil is not expected to be significant (SRC, 1989).

Briggs (1981) evaluated the soil/water distribution pattern of various organic chemicals in four types of silt-loam soils using initial chemical concentrations of 5, 10, 15 and 20 ppm. Because the soil adsorption of nonionic chemicals involves the partitioning of the substance between an organic phase and a water phase, the soil adsorption coefficient (K_d) can be correlated to the partitioning coefficient for soil organic matter and water (K_{om}):

$$K_{om} = 100K_d/(\%\ om)$$

where % om is the percent organic matter in the soil (Briggs, 1981). The average log K_{om} for 3-nitroaniline was reported to be 1.49. This is equivalent to a log K_{oc} of 1.72 assuming a conversion factor of 1.724 as reported by Briggs (1981).

2.3.2. Biodegradation. Most studies have shown that 3-nitroaniline is not readily degraded by soil microorganisms. Bordeleau and Bartha (1972) reported that nitroanilines, including 3-nitroaniline, were not susceptible to transformation by partially purified extracts of the soil fungus Geotrichum candidum. Alexander and Lustigman (1966) reported no degradation of a 10 µg/mℓ solution of 3-nitroaniline by a soil inoculum even after 64 days of incubation in the dark at 25°C. Zeyer and Kearney (1983) reported that 3-nitroaniline, when used as a sole carbon source, was not degraded by a strain of Pseudomonas isolated from soil. Similar results were seen with 2-nitroaniline, but 4-nitroaniline was degraded by the bacteria. The only study to demonstrate a potential biodegradation of 3-nitroaniline is that by McCormick et al. (1976). In this study, nitro reduction of 3-nitroaniline was achieved with cell-free extracts of the bacterial anaerobe Veillonella alkalescens.

2.3.3. Transport. There is little information available on the transport of 3-nitroaniline in soils. Based on a log K_{om} of 1.49 (as reported by Briggs, 1981), it has been suggested that this compound should be moderately mobile in soil (U.S. EPA, 1985). However, binding of the compound with humic substances is expected to substantially reduce translocation within the soil column.

2.4. SUMMARY

The available data indicate that the environmental fate of 3-nitroaniline is controlled largely by its water solubility, low vapor pressure,

susceptibility to photodegradation, and by its affinity to bind with humic substances in soil and sediments. Thus, 3-nitroaniline is not expected to be a major atmospheric contaminant. In may be present in aqueous media, but probably only near point sources. Although it is not readily susceptible to biodegradation, its binding with humic substances in soils and sediments will probably limit its bioavailability.

3. EXPOSURE

3-Nitroaniline may be released to the environment through process and waste emissions resulting from its production or use as a chemical intermediate (SRC, 1989). Humans are most likely to come into contact with this substance in occupational exposure settings. Information on occupational exposure is limited. No 3-nitroaniline is manufactured in the United States but 167,000 pounds is imported (USITC, 1976). Occupational exposure will occur primarily through dermal absorption and inhalation of vapors (Beard and Noe, 1981).

3.1. WATER

Limited monitoring data for this compound are available. Zoeteman et al. (1980) detected 3-nitroaniline at a concentration of 0.1 µg/ℓ in samples taken from the Rhine River near Lobith in the Netherlands.

3.2. WASTEWATER EFFLUENTS

Specific information on the occurrence of 3-nitroaniline in wastewaters is limited to one report indicating that the compound was present at a concentration of 259.55 mg/ℓ in an effluent from an organic chemical plant (U.S. EPA, 1987). Unspecified isomers of nitroaniline, at a concentration of 270 µg/ℓ, were also reported to be present in the raw wastewater of a dye manufacturing plant (Games and Hites, 1977). After treatment, the effluent contained no detectible levels of nitroaniline. 4-Nitroaniline in the wastewater from a dye manufacturing plant was reported to be oxidized 97.3% during 2 hours of chlorination (Endyus'kin and Filippov, 1980). A similar oxidation reaction could be expected with 3-nitroaniline.

3.3. SOIL

Soil monitoring data for 3-nitroaniline were not found in the available literature as listed in Appendix A.

3.4. FOOD

Monitoring data for 3-nitroaniline in food products were not found in the available literature as listed in Appendix A.

3.5. SUMMARY

In the absence of adequate monitoring data, few conclusions can be reached concerning levels of exposure of the general public to 3-nitroaniline. However, information provided in the previous section would suggest that exposure levels will probably be minimal except possibly in occupational exposure situations.

4. ENVIRONMENTAL TOXICOLOGY

4.1. AQUATIC TOXICOLOGY

4.1.1. Acute Toxic Effects on Freshwater Fauna. Deneer et al. (1987) evaluated the toxicity and bioaccumulation potential of various nitrobenzene derivatives including 3-nitroaniline, on the guppy, Poecilia reticulata. The tests were conducted under static water conditions using a standard water with a hardness of 25 mg/ℓ as $CaCO_3$. The log of the 14-day LC_{50} for 3-nitroaniline was reported to be 2.57. The corresponding LC_{50} would therefore be 371.5 µmol/ℓ (51 mg/ℓ). Deneer et al. (1987) reported that the relationship between the acute toxicity and the partition coefficient for nitroanilines could best be described by the following equation:

$$-\log LC_{50} = 0.88 \log P - 3.83 \quad (r = 0.959)$$

Toxicity studies on 4-nitroaniline indicate that the LC_{50} for several species of fish falls in the range of 10-100 ppm (U.S. EPA, 1985). For this same isomer, the LC_{50} for Daphnia magna is 24 ppm, and the EC_{50} for immobilization is 2.5 ppm (Bringmann and Kuhn, 1977). The toxicity threshold has been reported to be 3.1 ppm as measured by effects on a population of the protozoan Uronema parduci (Bringmann and Kuhn, 1980).

4.1.2. Acute Toxic Effects on Saltwater Fauna. No pertinent information on the acute toxic effects of 3-nitroaniline to marine organisms was found in the literature listed in Appendix A.

4.1.3. Chronic Effects on Fauna.

4.1.3.1. TOXICITY -- No pertinent information on the long term toxic effects of 3-nitroaniline was found in the literature listed in Appendix A.

4.1.3.2. BIOACCUMULATION AND BIOCONCENTRATION -- Sakiya et al. (1988) studied the uptake of several benzene derivatives, including 3-nitroaniline, through the body surface and gill membranes of goldfish. In tests conducted

with a 1.0 mM concentration at pH 6.0 and 25°C, the mean absorption rate constant [$(min^{-1}\ g^{-1})x10^4$] for 3-nitroaniline was found to be 1.570 (S.E.M. 0.286) for the body surface and 1.577 (S.E.M. 0.234) for the gills. In other studies conducted on carp, Sasaki (1978) found that 3-nitroaniline had a low potential for bioaccumulation. The bioconcentration factor was less than 200 after 8 weeks of exposure to concentrations ranging from 0.0001-0.01 of the 48-hour TL_m.

The bioconcentration factor (BCF) for 3-nitroaniline can be estimated using log K_{ow} and the regression equation derived by Briggs (1981):

$$\log BCF = 0.87 \log K_{ow} - 0.62$$

Briggs (1981) reported a log K_{ow} of 1.39; therefore, the log BCF would be 0.589, and the BCF 3.89. Howard et al. (1976) calculated a BCF of 8 for 3-nitro- aniline, 12 for 4-nitroaniline, and 6 for 2-nitroaniline. These values indicate that in general nitroaniline has a low potential for bioaccumu- lation.

4.1.4. Effects on Flora. Kramer et al. (1986) evaluated the effect of various monosubstituted nitrobenzenes on the autotrophic growth of synchronous cultures of the green alga Chlorella vulgaris. The isoactive inhibitory value ($pc_{50} = -\log EC_{50}$, where EC_{50}, in mol/ℓ, is the concentration causing a 50% reduction in growth) for 3-nitroaniline was reported to be 3.14 and 3.10 for measurements made at wavelengths of 680 and 750 nm respectively. These values are equivalent to EC_{50} concentrations of 0.724 mmol/ℓ (100 mg/ℓ) and 0.794 mmol/ℓ (110 mg/ℓ), respectively.

4.2. TERRESTRIAL TOXICOLOGY

4.2.1. Effects on Fauna. Limited data are available on the toxicity of 3-nitroaniline to terrestrial animals. Schafer et al. (1983) reported that the acute oral LD_{50}s for the redwinged blackbird (Agelaius phoeniceus),

starling (Sturnus vulgaris), Japanese quail (Coturnix coturnix japonica), and house sparrow (Passer domesticus) were 133 mg/kg, >1000 mg/kg, 562 mg/kg, and >1000 mg/kg, respectively. In another study, Schafer and Bowles (1985) evaluated the acute oral toxicity and repellency of various chemicals to house and deer mice. In a 3-day feeding study with 3-nitroaniline, less than 50% mortality of deer mice was recorded following an estimated daily oral dose >375 mg/kg. The compound was applied as a 2% solution to white wheat seeds included in the diet of the mice.

4.2.2. Effects on Flora. No pertinent data on the effects of 3-nitroaniline on plants were found in the literature listed in Appendix A.

4.3. FIELD STUDIES

No pertinent field data on the effects of 3-nitroaniline could be found in the literature cited in Appendix A.

4.4. AQUATIC RISK ASSESSMENT

Sufficient information is not available for a quantitative environmental risk assessment for 3-nitroaniline. Data are inadequate for deriving water quality criteria. Water Quality Criteria are based on a Criterion Maximum Concentration and a Criterion Continuous Concentration. The first value is equal to one-half of the Final Acute Value. The second criterion is equal to the lowest of the Final Chronic Value, the Final Plant Value, or the Final Residue Value. For 3-nitroaniline there is only one specific data point for acute toxicity and no information on which to derive a Criterion Continuous Concentration.

From a qualitative point of view, the available data on environmental persistence, toxicity, and bioaccumulation potential of 3-nitroaniline would suggest that the chemical represents only a low level of environmental risk.

Releases to the environment would be subject to physical and chemical degradation. Although rates of biodegradation are probably low, binding of the chemical with humic substances in soil and sediments is likely to limit its transport and bioavailability.

4.5. SUMMARY

Information on the environmental toxicology of 3-nitroaniline is limited. The available data for this isomer as well as for 4-nitroaniline indicate that acute median lethality to freshwater fish falls in the range of 10-100 mg/ℓ. There is no information on acute toxicity to saltwater species, or on chronic effects to either marine or freshwater organisms. The partition coefficient for 3-nitroaniline and experimentally derived and calculated bioconcentration factors indicate a low potential for bioaccumulation. Data for terrestrial vertebrates (birds and mice) indicate a relatively low acute toxicity of 3-nitroaniline, the oral LD_{50} values being above 100 mg/kg.

Sufficient information is not available for a quantitative environmental risk assessment for 3-nitroaniline. From a qualitative point of view, the available data on environmental persistence, toxicity, and bioaccumulation potential of 3-nitroaniline would suggest that the chemical represents only a low level of environmental risk.

5. PHARMACOKINETICS

5.1. ABSORPTION

Beard and Noe (1981) reported that 3-nitroaniline is readily absorbed through the lungs and also through intact skin. Other pertinent data regarding the absorption of 3-nitroaniline were not located in the sources searched (see Appendix A).

5.2. DISTRIBUTION

Pertinent data on the tissue distribution of 3-nitroaniline were not located in the sources searched (see Appendix A).

5.3. METABOLISM

3-Nitroaniline injected i.p. into rabbits or dogs appears in the urine as both conjugated and unconjugated forms of the parent molecule. The urinary substances were identified by the melting points of crystals obtained from ether extracts of urine before and after sulfuric acid hydrolysis (Wells et al., 1920-1921). Corbett and Corbett (1985) demonstrated that 3-nitroaniline undergoes N-oxidation to hydroxylamine and nitroso metabolites in the presence of hydrogen peroxide and chloroperoxidase using a cell-free system. Greater quantities of the hydroxylamine metabolite was formed than the nitroso metabolite. No C-oxidation products were found. The reaction was first order at substrate concentrations ranging from 0.05-0.20 mM, and the rate constant was 0.19 $m\ell \cdot \mu g^{-1}$ protein$\cdot min^{-1}$. Corbett and Corbett (1985) also demonstrated that 4-nitroaniline and 4-chloroaniline undergo N-oxidation under the same conditions. Rickert (1987) reported that 3-nitroaniline may be an intermediate for ~ 66% of the metabolites excreted into the urine of rabbits dosed with 1,3-dimethylbenzene, suggesting that 3-nitroaniline can be metabolized. These possible metabolites were identified as 4-amino-2-nitrophenol, 2-amino-4-nitrophenol,

1,3-diaminobenzene, and 2,4-diaminophenol. 3-Nitroaniline also is a metabolite of 1,3-dinitrobenzene in the rat (Bailey et al., 1988).

5.4. EXCRETION

Rabbits (1.87-2.13 kg) injected i.p. with 0.53 g of 3-nitroaniline excreted 13.7-15.6% of the dose into urine, 74-93% of which was excreted within the first 24 hours. 3-Nitroaniline was detected in the urine within 3 or 4 hours after injecting two dogs (6.3 and 10.5 kg) with 0.53 or 1.05 g, respectively, of 3-nitroaniline (lethal doses) (Wells et al., 1920-1921). Watanabe et al. (1976) reported that rats receiving 3-nitroaniline i.p. at 100 μmol/kg excreted diazo-positive metabolites into their urine within 5 hours after injection. The presence of diazo-positive substances in urine is an indicator for the presence of nitro-amino derivatives of benzene and to chlorobenzenes.

5.5. SUMMARY

3-Nitroaniline is readily absorbed through the lung and from intact skin. Data regarding absorption from the gastrointestinal tract and tissue distribution were not found. One report suggested that 3-nitroaniline is excreted into urine as conjugated or unconjugated forms of the parent molecule (Wells et al., 1920-1921); another report presented evidence that diazo-positive substances (indicators for the presence of nitro-amino derivatives) are excreted into urine of animals injected i.p. with 3-nitroaniline (Watanabe et al., 1976). The evidence also suggested that 3-nitroaniline, which is formed as a metabolite of 1,3-dinitrobenzene, is in turn metabolized to 4-amino-2-nitrophenol, 2-amino-4-nitrophenol, 1,3-diaminobenzene, and 2,4-diaminophenol (Rickert, 1987); another study showed that 3-nitroaniline can be metabolized to N-oxidation products in a cell free system (Corbett and Corbett, 1985).

6.1. SYSTEMIC TOXICITY

6.1.1. Subchronic Exposure. Pertinent data regarding the toxicity of 3-nitroaniline administered subchronically by the inhalation or oral routes were not located in the sources searched (see Appendix A).

6.1.2. Chronic Exposure. Pertinent data regarding the toxicity of 3-nitroaniline administered chronically by the inhalation or oral routes were not located in the sources searched (see Appendix A).

6.1.3. Other Relevant Information. Toxicity data for 3-nitroaniline are few. Oral LD_{50}s reported for rats are 535 mg/kg (RTECS, 1989), 540 mg/kg (Vernot et al., 1977), and 900 mg/kg (Vasilenko et al., 1974). The LD_{50} values reported for mice are 308 mg/kg (RTECS, 1989) and 310 mg/kg (Vernot et al., 1977), and the oral LD_{50} reported for guinea pigs is 450 mg/kg (Vernot et al., 1977).

Wells et al. (1920-1921) reported that a dog weighing 6.3 kg can be killed by injecting 15 mℓ (i.p.) of a 3.5% solution of 3-nitroaniline in olive oil (70 mg/kg as reported by the authors). [Using the value reported by the authors for the weight-normalized dose, the 3.5% solution in olive oil contained only 29.4 mg of 3-nitroaniline/mℓ; this concentration was used to calculate weight-normalized doses not reported by the authors]. In addition, Wells et al. (1920-1921) reported that rabbits weighing 1.7-2 kg are usually killed by 20 mℓ of 3-nitroaniline injected i.p. (calculated dose = 294-346 mg/kg), and 30 mℓ always kills rabbits weighing 1.7-2 kg (calculated dose = 400-519 mg/kg). A cat weighing 2.025 kg can be killed by 15 mℓ (calculated dose = 218 mg/kg) injected i.p., and a guinea pig weighing 0.97 kg can be killed by 7 mℓ (calculated dose = 212 mg/kg). A single

i.p. injection of 15 mℓ (200-250 mg/kg as reported by the authors) or 11 daily subcutaneous injections of 10 mℓ (calculated dose = 155 mg/kg) did not cause toxic effects in the rabbits.

The clinical effects of lethal doses of 3-nitroaniline in dogs are dyspnea and convulsion before death, and postmortem examination may show signs of asphyxia and dark-colored, slowly coagulating blood (Wells et al., 1920-1921). Repeated sublethal doses to rabbits cause profound emaciation and severe secondary anemia. Red and white blood cell counts may be decreased in rabbits given a single subcutaneous injection, and a second injection given 2 days after the first may cause a greater decrease in red and white blood cell counts, the appearance of numerous atypical white blood cells, bone marrow changes indicative of anemia, and swollen and dark-colored spleen and kidneys. Microscopically, the renal tubules may be distended and contain small globules or casts, and fatty degeneration may be seen in the epithelium of the straight tubules in Henle's loop (rabbits and dogs). Other microscopic effects reported by Wells et al. (1920-1921) were necrosis and fatty degeneration in the central lobular region in the liver, fatty degeneration in the myocardium, a distended spleen containing blood pigment, leucocytosis, and pulmonary edema.

3-Nitroaniline (4-nitroaniline also) is also a methemoglobin former in dogs and cats, and it causes hemolysis and Heinz body formations in dogs (DeBruin, 1976; Beard and Noe, 1981). Prolonged or excessive exposure may cause liver damage (DeBruin, 1976). The vapors of 3-nitroaniline are reported to be highly toxic (Beard and Noe, 1981). Vasilenko et al. (1974) and Vasilenko and Zvezdai (1974) reported that 3-nitroaniline is hematotoxic and causes significant increases in methemoglobin and sulfhemoglobin levels

in blood of mice and rats. Watanabe et al. (1976) reported that methemoglobinemia was seen 5 hours after Wistar rats were injected i.p. with 3-nitroaniline at 100 µmol/kg bw. Serum GOT and GPT activities were measured and were not found to be altered by treatment.

6.2. CARCINOGENICITY

6.2.1. Inhalation. Pertinent data regarding the carcinogenicity of 3-nitroaniline administered by the inhalation route were not located in the sources searched (see Appendix A).

6.2.2. Oral. Pertinent data regarding the carcinogenicity of 3-nitroaniline administered by the oral route were not located in the sources searched (see Appendix A).

6.2.3. Other Relevant Information. Although no data regarding the carcinogenicity of 3-nitroaniline were found in the literature, a qualitative evaluation of potential carcinogenicity can sometimes be based on evidence for structural analogues or metabolites. No data regarding the effects of long-term exposure to the isomers, 2- and 4-nitroaniline were found in the literature. Possible metabolites of 3-nitroaniline (based on indirect evidence) are 2,4-diaminophenol, 1,3-diaminobenzene, 4-amino-2-nitrophenol, and 2-amino-4-nitrophenol (Rickert, 1987). A brief summary of data regarding carcinogenicity and genotoxicity of these metabolites is presented below.

IARC (1987) classified 3-phenylenediamine (1,3-diaminobenzene) as a Group 3 carcinogen (not classifiable as to its carcinogenicity to humans), based on no data for humans and inadequate data for animals. Genotoxicity tests have shown positive results for 1,3-diaminobenzene in Salmonella in the presence of S9 (Garner and Nutman, 1977; Thompson et al., 1983).

In a 2-year feeding bioassay using male and female F344 rats and B6C3F1 mice, 4-amino-2-nitrophenol was carcinogenic in male rats, inducing transitional-cell carcinomas of the urinary bladder, possibly carcinogenic in female rats, but not carcinogenic in male and female mice (NCI, 1978). Commercial-grade 4-amino-2-nitrophenol induced mutations in Salmonella (Garner and Nutman, 1977, Dunkel and Simmon, 1980, Shahin et al., 1982), whereas a purified preparation (98% pure) did not induce mutations in Salmonella strains TA98, TA1537, TA1538, TA1535 and TA100 (Shahin et al., 1982; Shahin, 1985). NTP (1988), however, reported that 4-amino-2-nitrophenol (99.6% pure) induced mutations in Salmonella in the absence of S9 and forward mutations in mouse lymphoma cells. 4-Amino-2-nitrophenol did not induce unscheduled DNA synthesis in rat hepatocytes (Williams et al., 1982) and did not induce dominant lethal mutations (species not specified) (Burnett et al., 1977).

2-Amino-4-nitrophenol showed "some evidence of carcinogenicity" in male F344 rats administered the compound by gavage for 2 years (NTP, 1988). The incidences of renal cortical (tubular cell) adenomas and renal tubular cell hyperplasia were increased. 2-Amino-4-nitrophenol was not carcinogenic in female F344 rats or in male and female B6C3F1 mice (NTP, 1988). Shahin (1985) reported that 2-amino-4-nitrophenol induced mutation in Salmonella strains TA98 and TA1538, but only in the absence of S9. According to NTP (1988), 2-amino-4-nitrophenol induced mutations in Salmonella in the presence of S9, and in mouse lymphoma cells in the absence of S9. NTP (1988) also reported that 2-amino-4-nitrophenol induced sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells with and without S9.

6.3. GENOTOXICITY

The genotoxicity data for 3-nitroaniline are summarized in Table 6-1.

Garner and Nutman (1977) tested 3-nitroaniline along with azo dyes and other nitroaniline and nitrobenzene compounds for mutagenic activity in Salmonella typhimurium strain TA1538 using the soft agar overlay method. 3-Nitroaniline was mutagenic at 50 and 100 µg/plate only in the presence of rat liver S9. The other isomers, 2- and 4-nitroaniline, were also mutagenic in the presence of S9.

Chiu et al. (1978) showed that 3-nitroaniline induced mutations in Salmonella strain TA98 at a concentration of 10 µmol/plate (1380 µg/plate) in the absence of S9, but mutations were not induced at 0.1 and 1.0 µmol/plate. Under the same conditions, 3-nitroaniline was not mutagenic in TA100, and 2-nitroaniline and 4-nitroaniline were not mutagenic in either TA98 or TA100. Chiu et al. (1978) stated that Salmonella strains TA98 and TA100 have aerobic nitroreductase activity, and the liver nitroreductase activity (if present in S9) requires anaerobic conditions. Therefore, some caution must be exercised in interpreting these results, because it is possible that activation of 3-nitroaniline took place in the absence of S9. 3-Nitroaniline was mutagenic in only one of the Salmonella strains having the nitroreductase activity, and the 2- and 4-isomers were not mutagenic in either strain. Therefore, under these conditions, if the bacterial nitroreductase activates nitroanilines, it only activates the 3-isomer in only one Salmonella strain.

3-Nitroaniline was tested for mutagenic activity in eight Salmonella strains and in two E. coli strains using the gradient plate method and for DNA damaging activity in the unscheduled DNA synthesis assay using rat hepatocytes (Thompson et al., 1983). 3-Nitroaniline at concentrations

TABLE 6-1

Mutagenicity Testing of m-Nitroaniline

Assay	Indicator/ Organism	Application	Concentration or Dose	Activating System	Response/Comment	Reference
Reverse mutation	S typhimurium strains TA98, TA100, TA1538	plate (soft agar overlay method)	50 and 100 µg/plate	S9; phenobarbitone-induced rat liver	Positive at both concentrations in TA1538 with S9; negative without S9	Garner and Nutman, 1977
Reverse mutation	S typhimurium strains TA98, TA100	pour-plate method	0.1, 1.0, 10 µmol/plate	no activating system used	Positive in TA98 at 10 µmol; negative in TA100	Chiu et al., 1978
Reverse mutation	S typhimurium strains G46, TA1535, TA100, C3076, TA1537, D3052, TA1538, TA98	gradient plate method	≤1000 µg/mL of agar	S9; Aroclor 1254-induced rat liver	Positive in TA1535, TA100, TA1538 and TA98 30-100 µg/mL of agar with S9	Thompson et al., 1983
Reverse mutation	S typhimurium strains TA98, TA1537, TA1538, TA1535, TA100	plate incorporation	5-1000 µg/plate	S9; Aroclor 1254-induced rat liver	Positive in TA98, TA1538 and TA1535 with and without S9; negative in TA1537 and TA100	Shahin, 1985
Reverse mutation	S typhimurium strains TA98, TA1538, TA1537, TA100, TA1535	pour-plate method	0.05-10 mg/plate (6 concentrations)	S9; PCB-induced rat liver	Positive in TA98 (0.1-5 mg/plate), TA1538 (0.1-5 mg/plate), and TA100 (5 mg/plate) without S9; not tested with S9; negative in TA1537 and TA1535	Shimizu and Yano, 1986
Reverse mutation	S typhimurium strains TA98, TA100	preincubation method	100-5000 µg/plate	S9; PCB-induced rat liver	Positive in both strains with and without S9; greater response with S9 and in TA98	Kawai et al., 1987
Reverse mutation	S typhimurium strains TA98, TA100	plate incorporation, preincubation with or without FMN	0.05, 0.1, 0.15 and 0.2 µmol/plate	S9; Aroclor 1254-induced hamster or rat liver	Positive in TA98 with preincubation with FMN and with rat or hamster S9; response greater with hamster S9; negative with preincubation without FMN and with hamster or rat S9	Dellarco and Prival, 1989

TABLE 6-1 (cont.)

Assay	Indicator/ Organism	Application	Concentration or Dose	Activating System	Response/Comment	Reference
Reverse mutation	E. coli strains WP2, WP2uvrA-	gradient plate method	≤1000 µg/mℓ of agar	S9; Aroclor 1254-induced rat liver	Negative in both strains	Thompson et al., 1983
Kada Rec assay	B. subtilis H17 (argA14 trpB3, Rec$^+$), M45 (argA trpB3, rec-45, Rec$^-$)	plate	0.05-10 mg/plate (6 concentrations)	NR	Weak positive response	Skimizu and Yano, 1986
Unscheduled DNA synthesis	rat hepatocytes	cell culture	0.5-1000 nmol/mℓ (8 concentrations)	no activating system	Negative	Thompson et al., 1983

NR = Not reported

ranging from 30-100 µg/mℓ of agar was positive in the presence of S9 in strains TA1535, TA98, TA1538 and TA100, but not in strains G46, C3076, TA1537 and D3952. 3-Nitroaniline was negative in all Salmonella strains in the absence of S9, in E. coli strains WP2 and WP2uvrA- under all conditions, and in the unscheduled DNA synthesis assay. 4-Nitroaniline was positive in D3052, TA1538 and TA98 at concentrations ranging from 1-300 µg/mℓ of agar, and 2-nitroaniline was negative in all strains.

Shahin (1985) tested 3-nitroaniline (98% pure) at 5-1000 µg/plate and showed positive results in Salmonella strains TA98, TA1538 and TA1535 in the presence and absence of S9, but not in strains TA1537 and TA100 under the same conditions. In the absence of S9, mutations were induced in TA1535 at 500 and 1000 µg/plate, but only at 1000 µg/plate in the other positive strains, whereas in the presence of S9, mutations were induced at 250-1000 µg/plate in strain TA1535 and at 500 and 1000 µg/plate in the other positive strains. The number of revertants/µmol ranged from 8.2 (TA1537 without S9) to 30 (TA98 with S9).

Using the pour-plate method, Shimizu and Yano (1986) tested 3-nitroaniline at concentrations ranging from 0.05-10 mg/plate. The compound was mutagenic in Salmonella strains TA98, TA1538, and TA100 and negative in TA1537 and TA1535 in the absence of S9. The compound was not tested in the presence of S9. 3-Nitroaniline, at concentrations of 0.5, 1.0, and 5.0 mg/plate induced 25, 39, and 29 revertants/µmol, respectively. 3-Nitroaniline induced a slight positive response in the Kada B. subtilis rec assay (Shimizu and Yano, 1986).

Kawai et al. (1987) tested a series of aliphatic and aromatic nitro compounds, including 3-nitroaniline, for mutagenic activity in Salmonella strains TA100 and TA98 using the preincubation method. 3-Nitroaniline was

positive in strain TA100, inducing 90 revertants/mg of test compound in the presence of S9 and 230 revertants/mg in the absence of S9. Using strain TA98, 3-nitroaniline induced 1250 revertants/mg in the presence of S9 and 270 revertants/mg in the absence of S9. Therefore, 3-nitroaniline had about the same potency in both strains in the absence of S9, but was significantly more potent in TA98 in the presence of S9.

Dellarco and Prival (1989) used a preincubation method in which FMN was added to the preincubation mixture along with S9 to promote the reduction of the nitro group. Salmonella strains TA98 and TA100 were used, and the S9 fraction was obtained from Aroclor 1254-induced rat or hamster liver. 3-Nitroaniline was tested at 0.05-0.2 µmol/plate (6.9-27.6 µg/plate). Data were not presented for strain TA100, but the authors reported that the mutagenic activity was greater in TA98 than in TA100. 3-Nitroaniline was strongly mutagenic using the FMN preincubation protocol in the presence of hamster S9, but was less potent in the presence of rat S9. No mutagenic activity was observed without FMN in the presence of hamster or rat S9. A rough estimate showed that 3750, 6167, and 8375 revertants/µmol of 3-nitroaniline were induced at concentrations of 0.1, 0.15, or 0.2 µmol, respectively, in the presence of hamster S9, whereas only 1500, 2167, and 3500 revertants/µmol, respectively, were induced at the same concentrations in the presence of rat S9. These results showed that 3-nitroaniline was a more potent mutagen when FMN was incorporated into the assay mixture, indicating that nitro reduction may indeed be involved in the activation of 3-nitroaniline.

The mutagenicity studies discussed above have shown that under conditions in which a bacterial nitroreductase could have activated 3-nitroaniline (mutagenic activity in the absence of S9), high concentrations of

3-nitroaniline were generally required to induce revertants. In one study, 3-nitroaniline at a concentration of 10 µmol or 1380 µg/plate was mutagenic in the absence of S9 (Chiu et al., 1978), and in another study, it was mutagenic at 500-5000 µg/plate in the absence of S9 (Shimizu and Yano, 1986). 3-Nitroaniline tested at 10-5000 µg/plate induced 2.6-fold more revertants in TA100 in the absence of S9 than in the presence of S9, whereas 3-nitroaniline was more potent in TA98 in the presence of S9 than in the absence of S9 (Kawai et al., 1987), indicating that, if bacterial nitroreductase is involved in activating 3-nitroaniline, some strains have more activity than others. At very low concentrations of 3-nitroaniline (6.9-27.6 µg/plate), FMN, in addition to S9, was required for mutagenic activity.

6.4. DEVELOPMENTAL TOXICITY

Pertinent data regarding the developmental toxicity of 3-nitroaniline were not located in the sources searched (see Appendix A). Some possible metabolites of 3-nitroaniline include 4-amino-2-nitrophenol, 2-amino-4-nitrophenol, 1,3-diaminobenzene and 2,4-diaminophenol (Rickert, 1987). The literature on these metabolites was searched for evidence of developmental toxicity. Two studies of the developmental toxicity of 1,3-diaminobenzene were located. Picciano et al. (1983) treated mature female Sprague-Dawley rats by gavage with 1,3-diaminobenzene at 0 (propylene glycol vehicle control), 45, 90 or 180 mg/kg bw/day on days 6-15 of gestation. The day that a vaginal plug or spermatozoa in the vaginal smear was found was designated day 0 of gestation. Positive controls (vitamin A and aspirin) were maintained. Pregnant dams (22, 9, 8 and 7 in vehicle control, 45, 90 and 180 mg/kg bw/day groups, respectively) were killed and reproductive tracts and contents were examined on gestation day 20. No

deaths occurred, and no signs of maternal toxicity were observed, except that high-dose rats exhibited a significantly ($p<0.05$) reduced rate of body weight gain. Parameters of fertility and fetal body weights were unaffected by treatment. The incidences of gross external skeletal and visceral anomalies were not increased by exposure to 1,3-diaminobenzene. Positive controls responded appropriately.

A German paper reported the results of a developmental toxicity study of 1,3-diaminobenzene (Hruby et al., 1981). Groups of 25 mated female rats were treated by gavage with 1,3-diaminobenzene at 0 (water control), 10, 30 or 90 mg/kg bw/day on days 6-15 of gestation. In addition, a positive control group was treated with acetylsalicylic acid. Six high-dose rats died; no rats in the other groups died. Evidence of fetotoxicity was noted in high-dose rats; a smaller number of litters with live fetuses, reduced placental weight, fewer living fetuses/litter, reduced fetal body weights, increased incidence of fetal resportion and a greater number of dead fetuses were observed. Major fetal malformations were not observed in treated rats, but an increase in the percentage of litters with minor fetal abnormalities and a greater percentage of fetuses with minor abnormalities were noted in high-dose rats, compared with negative controls. These signs of fetotoxicity and fetal anomalies were not statistically significant in the 10 or 30 mg/kg bw/day dosed groups. Data were unavailable for the remaining metabolites.

6.5. OTHER REPRODUCTIVE EFFECTS

Pertinent data regarding other reproductive effects of 3-nitroaniline were not located in the sources searched (see Appendix A).

6.6. SUMMARY

With the exception of genotoxicity data, almost all toxicity data were related to acute exposure. Lethality data for 3-nitroaniline showed LD_{50}s of 308 mg/kg for mice, 450 mg/kg for guinea pigs, and 535-900 mg/kg for rats (Vernot et al., 1974; Vasilenko et al., 1974; RTECS, 1989). Other data showed that dogs can be killed by a single i.p. injection of 70 mg/kg, guinea pigs by 212 mg/kg, cats by 218 mg/kg, and rabbits by 294-346 mg/kg (Wells et al., 1920-1921. A single i.p. injection of 200 mg/kg is without toxic effects in the rabbit (Wells et al., 1920-1921).

Clinical effects from acute exposure to 3-nitroaniline include dyspnea and convulsions before death, with postmortem signs of asphyxia (Wells et al., 1920-1921). 3-Nitroaniline is a methemoglobin and a sulfhemogloben former (DeBruin, 1976; Beard and Noe, 1981; Watanabe et al., 1976; Vasilenko et al., 1974; Vasilenko and Zveydai, 1974). Other toxic effects may include decreases in red and white blood cell counts, bone marrow changes indicative of anemia, pulmonary edema, and damage to the kidney, spleen, liver, and heart (Wells et al., 1920-1921). Serum GOT and GPT activities in rats were not affected by i.p. doses of 100 µmol/kg (Watanabe et al., 1976).

No data were available regarding subchronic or chronic toxicity, carcinogenicity, teratogenicity, or reproductive toxicity of 3-nitroaniline.

Genotoxicity data showed that 3-nitroaniline is mutagenic in Salmonella typhimurium under various conditions. It has produced positive results in strains capable of detecting both base-pair substitutions (TA100 and TA1535) and frameshift mutations (TA98, TA1537 and TA1538). In general, concentrations $\geq$500 µg/plate are required to induce mutations in the absence of S9 (Chiu et al., 1978; Shahin, 1985; Shimizu and Yano, 1986). Lower concentrations (30-250 µg/plate) of 3-nitroaniline can induce mutations in the

presence of S9 (Garner and Nutman, 1977; Thompson et al., 1983). 3-Nitroaniline preincubated with FMN in the presence of hamster or rat liver S9 can induce mutations at concentrations as low as 6.9-27.6 µg/plate (Dellarco and Prival 1989). This study indicates that nitroreduction may be a factor in the activation of 3-nitroaniline. Other genotoxicity tests showed that 3-nitroaniline induced a weak positive response in the Kada rec assay (Shimizu and Yano, 1986) and a negative response in the test for unscheduled DNA synthesis using rat hepatocytes (Thompson et al., 1983).

Analysis of possible metabolites of 3-nitroaniline showed that data were inadequate for evaluating the carcinogenicity of 1,3-diaminobenzene; 4-amino-2-nitrophenol and 2-amino-4-nitrophenol were carcinogenic in male rats, but not in male and female mice. 4-Amino-2-phenol was possibly carcinogenic in female rats and 2-amino-4-nitrophenol was not carcinogenic in female rats. All three compounds were mutagenic in Salmonella in the presence or absence of S9.

Analysis of possible metabolites has shown that 1,3-diaminobenzene is fetotoxic and demonstrates significant developmental toxicity at 90 mg/kg bw/day when given during days 6-15 of gestation (Hruby et al., 1981).

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

There are no U.S. EPA regulatory or verified guidance values currently available for human exposure to 3-nitroaniline by either oral, inhalation or dermal routes.

No occupational exposure standards for 3-nitroaniline have been established by OSHA, and no occupational exposure recommendations have been made by ACGIH or NIOSH. However, OSHA (1989) has established an 8-hour TWA for 4-nitroaniline of 3 mg/m^3 (with a skin notation). This level is identical to the TLV® currently recommended by ACGIH (1989).

7.2. AQUATIC

There are no standards or guidelines currently available for the protection of water resources and aquatic life from exposure to 3-nitroaniline. There are also no standards or guidelines currently available for the other isomers of nitroaniline.

8. RISK ASSESSMENT

Statements concerning available literature in this document refer to published, quotable sources and are in no way meant to imply that confidential business information (CBI), which this document could not address, does not exist. From examination of the bibliographies of the CBI data, however, it was determined that CBI data that would alter the approach to risk assessment values presented herein do not exist.

8.1. CARCINOGENICITY

Data were not available for a qualitative evaluation of the potential carcinogenicity of 3-nitroaniline by any route of exposure.

8.1.1. Weight of Evidence. No data were available regarding carcinogenicity of 3-nitroaniline, and no inferences can be made regarding the two isomeric forms, 2- or 4-nitroaniline, because long-term studies have not been conducted. Possible metabolites (based on indirect evidence) include 4-amino-2-nitrophenol, 2-amino-4-nitrophenol, 1,3-diaminobenzene, and 2,4-diaminophenol. IARC (1987) classified 1,3-diaminobenzene as a Group 3 carcinogen, and NCI (1978) and NTP (1988) concluded that 4-amino-2-nitrophenol and 2-amino-4-nitrophenol were carcinogenic in male rats, but not in male and female mice and that 4-amino-2-nitrophenol was possibly carcinogenic in female rats and 2-amino-4-nitrophenol was not carcinogenic in female rats. All three compounds were mutagenic in bacteria; 4-amino-2-nitrophenol and 2-amino-4-nitrophenol were mutagenic in mammalian cells; and 2-amino-4-nitrophenol was also clastogenic. Because there are no definitive data showing that these compounds are metabolites of 3-nitroaniline and the data showed only limited evidence of carcinogenicity (positive in only one sex of one species) for these possible metabolites, there is insufficient evidence to modify the over-all-weight of evidence group for 3-nitroaniline.

Therefore, according to EPA methodology for carcinogen risk assessment (U.S. EPA, 1986a), 3-nitroaniline is classified as weight-of-evidence Group D, not classifiable as to human carcinogenicity.

8.1.2. Quantitative Risk Estimates. Data were not available for a quantitative evaluation of the potential carcinogenicity of 3-nitroaniline.

8.2. SYSTEMIC TOXICITY

Data were not available for assessing the systemic toxicity of 3-nitroaniline by the inhalation or oral route for less than lifetime or chronic exposure.

9. REPORTABLE QUANTITIES

9.1. BASED ON SYSTEMIC TOXICITY

No data regarding subchronic or chronic toxicity of 3-nitroaniline were found in the literature; therefore, a chronic RQ for 3-nitroaniline cannot be derived.

9.2. BASED ON CARCINOGENICITY

3-Nitroaniline was classified as weight-of-evidence Group D. Based on EPA methodology for evaluating potential carcinogens for adjusting reportable quantities (U.S. EPA, 1986b), 3-nitroaniline does not receive a hazard ranking based on carcinogenicity.

10. REFERENCES

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