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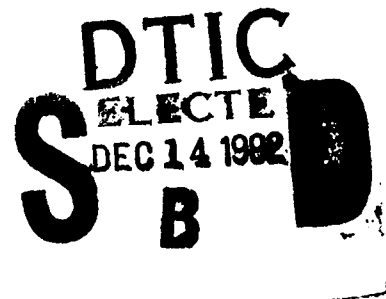
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TECHNICAL REPORT

RECOMMENDED WATER QUALITY CRITERIA FOR
OCTAHYDRO-1,3,5,7-TETRAZOCIN
(HMX)

HOWARD T. BAUSUM, Ph.D.



U S ARMY BIOMEDICAL RESEARCH & DEVELOPMENT LABORATORY

Fort Detrick

Frederick, MD 21701-5010

27 March 1989

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) is a colorless, crystalline nitramine used, in various formulations, in military munitions and rocket propellants. The compound is released to the environment in wastewaters resulting from manufacturing and loading processes. Health and environmental effects data were analyzed and reviewed. In tests with four species of freshwater invertebrates and four species of fish, HMX was not acutely or chronically toxic up to the limit of aqueous solubility, approximately 3.9 mg/L. In one report, toxic effects were encountered in post-hatching stages of the bluegill. In tests with four species of algae, toxic effects were not encountered. All tests reported with aquatic organisms were static, ranging up to 96 hrs; chronic or flow-through tests were not reported. HMX is poorly absorbed when administered orally to rats or mice; most (85% in rats, 70% in mice) was recovered in the feces. After intravenous administration of ¹⁴ C-HMX to					
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19. ABSTRACT (Continued)

the rat, 61 percent of the radioactivity was eliminated in the urine, six percent as CO₂, and only five percent retained in the body at four days. Plasma concentrations were lower than those in tissues; highest concentrations were observed in liver and kidney. There was rapid metabolism of part of the administered compound to very polar substances.

Adverse health effects were not found in persons exposed occupationally or experimentally to HMX, except for minimal erythematous lesions in skin patch tests. Oral LD₅₀ values were about 6.3 g/kg in mice and about 2.3 g/kg in rats. The central nervous system is a target system, with hyperkinesia, ataxia, and, at higher doses, convulsions observed. Histologic changes in the liver were important in short-term (14-day) studies in rats.

In a 13-week feeding study with mice, though mortality was encountered at doses of 200 to 750 mg/kg/day, gross or histopathologic changes and other toxic signs were not encountered, with minor, questionable, exceptions. In a companion 13-week study with rats, histologic changes in the liver were found in males (450, 1350, and 4000 mg/kg/day) and tubular kidney changes (focal atrophy and dilation) in females (270, 620, and 1500 mg/kg/day). A no-observed-adverse-effect level (NOAEL) of 50 mg/kg/day was determined.

No lifetime studies nor determinations of developmental toxicity, reproductive effects, or carcinogenicity were found. Tests of mutagenicity have been negative.

Environmental fate studies with HMX indicate that it can persist for great distances below a source, but that both photolysis and biodegradation can play a role in reducing residual levels. Wastewaters containing HMX can be successfully treated in biological systems under anaerobic conditions. Sensitive analytical methods exist for detection of HMX.

Due to limitations in the data base, it is not at present possible to derive water quality criteria for protection of aquatic life following USEPA guidelines. Based on the NOAEL of 50 mg/kg/day from the 13-week rat study, an allowable human daily intake (ADI) of 3.5 mg/day was calculated. An ambient water quality criterion of 1.7 mg/L was proposed for the protection of human health. This compares to a value of 0.103 mg/L proposed for RDX.

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1. INTRODUCTION

Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine is a nitramine explosive and propellant compound manufactured by the US Army. It is commonly called HMX (for High-melting Explosive). It is chemically similar to RDX, is made by a modification of the process used in RDX synthesis, and is present as a minor constituent in RDX. HMX is used in a variety of explosive, bonded explosive, and propellant formulations by the armed services and civilian manufacturers of rocket propellants.

The objectives of this report are to review existing data on HMX, with special reference to those on human, mammalian, and aquatic health effects, and to generate water quality criteria for drinking water and for the protection of aquatic life and its uses. For this purpose, USEPA methods will be followed; these are summarized in the appendices: Appendix A, derivation of criteria for the protection of aquatic life and its uses (Stephan et al. 1985), and Appendix B, for the protection of human health (USEPA 1980).

PHYSICAL AND CHEMICAL PROPERTIES (from Lindner, 1980, unless otherwise indicated)

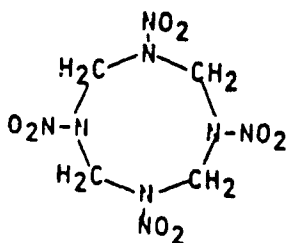
Common designation: HMX (for High Melting Explosive, Fedoroff and Sheffield, 1966); octogen.

Chemical name: Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine.

Synonyms: Cyclotetramethylenetetranitramine; tetramethylene tetranitramine; 1,3,5,7-tetraza-1,3,5,7-tetranitro-cyclooctane; homocyclonite.

Molecular formula: $C_4H_8N_8O_8$

Structural formula:



Molecular weight: 296.17

CAS Registry number: 2691-41-0

Elemental analysis: C, 16.22 percent; H, 2.72 percent; N, 37.84 percent; O, 43.22 percent.

Color: white

Crystal density, g/cm³: 1.90 (beta)

Crystal form: four polymorphic forms; beta form is least sensitive and most stable.

Vapor pressure: 3×10^{-9} mm Hg at 100°C

Melting point: 286°C

Hardness, Mohs: 2.3

Solubility, g/L: water - 0.0066 at 20°C, 0.14 at 83°C; acetone - 22.0 at 30°C; cyclohexanone - 53.0 at 30°C; acetic anhydride - 13.0 at 30°C (Patterson et al., 1977); dimethyl sulfoxide - soluble. Generally less soluble in a given solvent than RDX

Specific heat, J (=g X K): 1.26

Heat of formation, kJ/g: -0.253

Heat of combustion, kJ/g: 9.43

Heat of detonation, kJ/g: 5.67

Heat of vaporization, J/g: 368

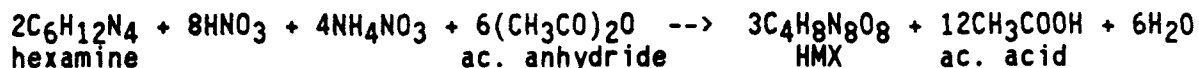
Oxygen balance, percent to CO₂: -22

Detonation products, experimentally determined in a bomb calorimeter, mole per mole HMX: 3.68 N₂; 3.18 H₂O; 1.92 CO₂; 1.06 CO; 0.97 C; 0.395 NH₃; 0.30 H₂. Other products include N₂O, HCN, CH₂O. Proportions vary with conditions.

MANUFACTURE AND USE

The Army is the only manufacturer of HMX in the United States. It is produced at Holston Army Ammunition Plant (AAP), Kingsport, Tennessee, operated by the Holston Defense Corporation, a subsidiary of Eastman Kodak. HMX is produced by a modification of the Bachman process (Bachman and Sheehan, 1949), which is also used in the manufacture of RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine). A description and flow diagrams of the process are given by Pal and Ryon (1986). This process involves the nitration of hexamine (hexamethylene tetramine) with nitric acid and ammonium nitrate in an acetic acid/acetic anhydride solvent. HMX production differs from that of RDX in the proportion of starting materials and in the optimal temperature (44°C for HMX). The processing steps, which are outlined by Kitchens(1979), are essentially the same and include simmering of the reaction mixture to minimize

by-products. In each case, formation of one nitramine is favored, but the final product contains a few percent of the other compound as well. The normal stoichiometric reaction for the formation of HMX is:



Production capacity for HMX is 15 million lb/yr, with actual production somewhat variable, perhaps 5 to 10 percent of capacity. Most HMX is used by the Army or Navy in formulating a number of explosive mixtures. The remaining HMX is sold to civilian manufacturers, including Hercules, Thiokol, and Aerojet General, for use in rocket propellant formulations. These propellants are produced for the military, thereby making HMX a military-unique compound (Kitchens, 1979).

ENVIRONMENTAL EFFECTS AND FATE

Wastewaters resulting from the manufacture and loading of HMX and HMX-containing propellants and explosives may be discharged to the environment. The greatest source for HMX release is found at Holston AAP and includes several steps in the manufacturing process, especially recrystallization and dewatering, and incorporation operations. In addition, load, assemble and pack (LAP) operations involving HMX-containing explosives and propellants take place at approximately seven AAPs and four naval facilities. HMX and RDX almost always occur together in AAP discharge water. This is due to HMX's being a contaminant of RDX manufacture and from their sharing a common manufacturing process (Sullivan et al., 1979). In studies of effluent wastewater at Holston AAP, Stidham (1979) calculated an average value for total HMX release to wastewater at 45 lbs per day (0.164 lb/ton of product), with concentrations ranging from 0.09 to 3.36 mg/L. Kitchens (1979) reported HMX concentrations of 0.01 mg/L, in the Holston River, to 2.6 mg/L in some wastewater samples. Only partial removal is accomplished by present treatment of the wastewaters.

2. AQUATIC TOXICOLOGY, TOXICITY TO PLANTS AND ANIMALS

TOXICITY TO AQUATIC ORGANISMS

Data available for calculating a water quality criterion for HMX do not meet all requirements specified by the USEPA guidelines (Stephan et al. 1985). However, since the available data are fairly consistent in their assessment of the toxicity of HMX, information on the effects of this compound on aquatic organisms is presented and discussed below.

Aquatic Invertebrates

Bentley et al. (1977) performed static acute toxicity tests on four species of freshwater invertebrates. These were Daphnia magna (water flea, a Cladoceran), Asellus militaris (sowbug, an isopod), Gammarus fasciatus (amphipod), and Chironomus tentans (midge) larvae. The tests were conducted for 24 and 48 hours at 20°C, pH 7.1, and 35 mg/L hardness. The EC50 values, based on immobilization at 48 hours, were all in excess of 32 mg/L, the highest level of HMX tested. No adverse effects on these test animals were reported. These results reflect a low acute toxicity, which may be considered lower than that for either algae or fish (Sullivan et al. 1979). It should be recognized, however, that only nominal concentrations were reported and that, due to solubility problems with this compound, the actual concentrations were considerably lower.

In a later study (Bentley et al. 1984), no lethality was observed in 24 and 48 hour static acute tests with D. magna at concentrations up to 3.9 mg/L, which was reported by the investigators to be the limit of aqueous solubility under the conditions used. Daphnid chronic toxicity tests were then undertaken (22°C, pH range 7.8-8.5), with percentage survival determined at weekly intervals to 28 days at mean measured HMX levels up to 3.9 mg/L. In all cases, survival was comparable to that of controls. Cumulative offspring per female were also determined at intervals throughout the 28-day study and showed no statistically significant reduction from those in the control group.

Fish

An evaluation of the toxic effect of exposure to wastewater from an AAP producing RDX and HMX was performed by Stilwell et al. (1977), using the fathead minnow, Pimephales promelas. The fish were exposed in a 96-hour static assay to various dilutions of the waste. The LC50 values ranged from 1 percent to 70 percent of the effluent concentrations, depending on the part of the plant from which the effluent was produced. HMX and RDX concentrations in the waste ranged up to about 6 ppm. There was a positive correlation between HMX content and toxicity.

The toxicity of HMX to four species of fish was determined by Bentley et al. (1977) in static acute tests of 24-, 48-, and 96-hour durations. They exposed bluegills (Lepomis macrochirus), fathead minnows, rainbow trout (Salmo gairdneri), and channel catfish (Ictalurus punctatus) to various

concentrations of HMX and found that in all cases LC50 values were over 32 mg/L nominal concentration. In further static tests with bluegills, temperature was varied from 15 to 25°C, pH from 6 to 8, and hardness from 35 to 250 mg/L as CaCO₃. Again all LC50 values were above 32 mg/L, showing that specific test conditions were not responsible for the low toxicity. In tests of various life stages of the fathead minnow, a greater degree of sensitivity was observed only for the 7-day posthatching stage, in which the LC50 was 25 mg/L in 48-hour tests and 15 mg/L for 96 hours.

Because of this potential toxicity to developing fry, the Army chose to test further the toxicity of HMX to fish in an embryo-larval study using the fathead minnow, *P. promelas* (Bentley et al. 1984). In preliminary static acute tests at nominal concentrations from 1.9 to 15 mg/L, no adverse effects were observed at exposures from 24 to 96 hours. In further tests (Table 1), fry were exposed for 32 days posthatch to HMX solutions up to the limit of aqueous solubility (measured concentrations: 0.22, 0.32, 0.78, 1.3, and 3.3 mg/L). Percentage hatch, percentage survival, and mean weight and length were in all cases comparable to the control values. This result was not in agreement with the earlier finding (Bentley et al., 1977) of toxicity to 7-day-old fry.

Table 1. Percentage hatch, survival, and mean length and weight of fathead minnow fry (*Pimephales promelas*) exposed to HMX for 32 days post-hatch in embryo-larval static tests (after Bentley et al., 1984). A and B are replicate tests.

mg/L HMX (measured)		Percent hatch	Percent survival	Mean length (mm)	Mean weight (g)
3.3	A	93	98	21(2.6)a	0.066
	B	95	98	21(2.2)	0.072
1.3	A	93	96	21(2.3)	0.068
	B	95	100	21(2.5)	0.063
0.78	A	100	95	21(1.9)	0.064
	B	98	97	20(2.4)	0.058
0.32	A	97	95	21(2.0)	0.064
	B	97	95	19(2.7)	0.054
0.22	A	98	100	20(2.5)	0.060
	B	93	100	20(2.7)	0.058
control	A	95	96	20(2.1)	0.062
	B	97	95	20(2.5)	0.060

a - standard deviation

It is apparent that HMX is not acutely or chronically toxic to the fish tested, or to invertebrates, at the limit of aqueous solubility. Although nonaqueous solvents were not used in these studies, extraordinary means were used to maximize solution, i.e., 1 week of stirring at 25°C.

Aquatic plants and Bacteria

The toxicity of HMX to algae was also studied by Bentley et al. (1977). Included were two species of blue-green algae, Microcystis aeruginosa and Anabaena flos-aquae, the green alga Selenastrum capricornutum, and the diatom Navicula pelliculosa. Nominal concentrations from 0.32 to 32 mg/L were employed in 96-hour static tests. Endpoints evaluated were cell density (cells per ml) and chlorophyll a content. Small increases in these parameters were seen in many cases, but these were not significantly different from controls, when probit transformations were used. In no case were any deleterious effects found, indicating that EC50 values are in excess of the highest concentrations tested. Sullivan et al. (1979) applied additional statistical treatment to these data and found some of the increases in cell density and chlorophyll a to be significant. They did not equate this with biological significance. No data were found for the effects of HMX on vascular aquatic plants. Nor were data found on the toxic effects on bacteria, except as noted in the section on mutagenesis.

TOXICITY TO TERRESTRIAL ORGANISMS

Data on mammals frequently used in laboratory experiments are given in another section. No other data were found on the toxic effects of HMX on terrestrial plants, invertebrates, birds or mammals.

3. PHARMACOKINETICS

ABSORPTION

In a study of the pharmacokinetics of HMX in rats and mice, Cameron (1986) gave a single oral dose of the ^{14}C -labelled compound (500 mg/kg) to rats (five animals per sex) and mice (five animals per sex). The HMX was prepared in aqueous carboxymethylcellulose solution and delivered by gavage. In both species, radioactivity was rapidly eliminated, mainly in the feces. Thus in rats 85 percent had been eliminated in the feces in 96 hours. In mice the corresponding figure was 70 percent. The remaining radioactivity was largely absorbed; amounts remaining in the gastrointestinal tract at 96 hours were 0.07 percent in the rat and 0.16 percent in the mouse. There appeared to be no significant differences in these values between the sexes.

Cameron (1986) also estimated systemic absorption of an oral dose in the rat by comparing percent of radioactivity appearing in the urine following oral and intravenous administration of ^{14}C -HMX. Results indicated that only about 6 percent of material administered p.o. was absorbed. A similar calculation was made based on peak plasma levels achieved following oral and i.v. administration. An estimate of only 4 percent absorption of the oral dose was obtained. This estimate is probably less reliable, because of the low plasma levels observed. It was concluded that in rats about 5 percent of an oral dose of HMX is absorbed into the systemic circulation. The above estimates of absorption from the gut could all be low if rapid secretion into the bile occurs.

Absorption of HMX through the skin is also poor, with large doses required to produce significant toxic effects. McNamara et al. (1974) applied HMX, 33 percent (w/v) in dimethylsulfoxide (DMSO), to the clipped backs of rabbits. At a dose of 1.0 mL (165 mg/kg), no changes in blood cell or blood chemistry parameters nor lesions in internal organs were noted. The actual skin area covered, in cm^2 , was not stated. In guinea pigs, similarly exposed to 1000 or 2000 mg/kg, no gross systemic effects were noted, although several deaths occurred and were attributed to HMX. The same authors reported an intravenous LD50 for guinea pigs of 28.2 mg/kg. Similar experiments were also performed by McNamara et al. (1974) on beagle dogs. There were no consistent changes in blood pressure, heart rate, respiratory rate, EKG, or EEG, nor abnormal responses to stimuli, over a four-week period following topical application of HMX, 289 or 480 mg/kg, in DMSO (treated area in cm^2 not stated). When the i.v. route was used, hypotension and a sleeplike EEG pattern were produced by doses as low as 2.5 mg/kg. Comparable results were obtained when acetone or cyclohexanone was used as the solvent, although the applied concentrations of HMX were lower. It was concluded that the skin of these animals is poorly penetrated by HMX.

Toxicity by the dermal route was also reported by Cuthbert et al. (1985) to be far lower than toxicity by the i.v. route. Thus in the rat, the intravenous LD50 was reported as 25 mg/kg for males and 38 mg/kg for females, while the values for dermal exposure were in excess of 5000 mg/kg. For

rabbits, i.v. LD50 was 10 to 15 mg/kg, while dermal LD50 was 670 mg/kg for males and 1340 mg/kg for females.

DISTRIBUTION AND EXCRETION

Cameron (1986) administered ^{14}C -HMX (500 mg/kg) to rats (five males and five females) as a single oral dose, given by gavage in aqueous hydroxymethylcellulose. After 96 hours, 85 percent had been eliminated in the feces, 4 percent in the urine. Only 0.7 percent remained in the gastrointestinal tract and carcass. In two animals, $^{14}\text{CO}_2$ was collected for 48 hours post dose. This represented 0.5 percent of the total radioactivity. There was apparently no difference between males and females in the results obtained. In a parallel experiment in mice, again 500 mg/kg, after 96 hours 70 percent had been eliminated in the feces and 3 percent in the urine. Only 0.6 percent was retained in the gastrointestinal tract and carcass. Again $^{14}\text{CO}_2$ was collected from two animals over the first 48 hours. This accounted for 1.1 percent of the total dose.

Levels of radioactivity in the plasma increased slowly during the first six hours post dose. These peaked in both species between 6 and 12 μg equiv/ml. If a plasma volume of 45 ml/kg is assumed, these peak concentrations accounted for only 0.07 percent of the total dose administered. In relation to administered dose, plasma levels were low and showed little change with increasing dose (Henderson, 1985b).

Cameron (1986) also studied the pharmacokinetics of radioactivity following single-dose intravenous administration of ^{14}C -HMX (2 mg/kg) to the rat (five animals of each sex). The dose was administered in 30 μl DMSO with injection into the saphenous vein. This was regarded as roughly representing the absorbed portion of an oral dose. After 96 hours, 61 percent had been eliminated in the urine and 3 percent in the feces, while 5 percent was retained in the intestinal tract and carcass. There appeared to be no difference in the results between males and females. From two animals, $^{14}\text{CO}_2$ was collected for the first 24 hours. This amounted to about 6 percent of the total radioactivity administered. The rates of excretion, in relation to the remaining body burden, were high only in early time periods. For example in the period from 72 to 96 hours post-dose, only 0.5 percent of the total dose was excreted, while 5 percent of the total was being retained in the body. Only 70-80 percent of the radioactivity was recovered. The fate of the remainder was not determined, although the author suggested that $^{14}\text{CO}_2$ excretion after the 48 hour mark, or excretion of other volatile metabolites, may have contributed to incomplete recoveries.

Plasma levels of total radioactivity in these rats increased during the first hour post-dose. They then tended to maintain a plateau for up to 6 hours at concentrations of 1 μg equiv/ml in males and 0.5 μg equiv/ml in females. In the period 6 to 72 hours, plasma radioactivity levels declined greatly, and levels in males and females were both approximately 0.2 μg equiv/ml at 24 hours and 0.05 μg equiv/ml at 72 hours. If a plasma volume of 45 ml/kg is assumed, then the peak concentrations, at one to six hours post-

dose, indicate that at that time 2.3 percent (males) or 1.1 percent (females) of the total dose was circulating in plasma. Following single i.v. administration of ^{14}C -HMX, (2 mg/kg) to 6 male and 6 female rats, radioactivity was measured in various tissues and organs of individual animals sacrificed at 2 min, 24 hours, and 96 hours post-dose (Cameron 1986). At 2 min, highest levels were observed in lung and heart and lowest levels in the brain. (Table 2). At 24 and 96 hours post-dose, highest concentrations were found in liver and kidney. Total recoveries in the rat body were approximately 90 percent at 2 min, 17 percent at 24 hours, and 5 percent at 96 hours. Tissue to plasma ratios of radioactivity, shown in Table 3, generally increased with time. An exception is the 2 min value for lung, which is the first tissue to encounter an i.v.-administered chemical.

Table 2. Radioactivity in various tissues at intervals following a single i.v. dose of ^{14}C -HMX (2 mg/Kg) to 6 male and 6 female rats.

Tissue	Radioactivity ($\mu\text{g equiv/gm}$)		
	2 min	24 hr	96 hr
Whole blood	2.15	0.22	0.07
Plasma	1.58	0.18	0.05
Lung	15.39	0.32	0.19
Liver	4.25	0.83	0.26
Kidney	3.96	0.74	0.36
Skeletal muscle	1.28	0.22	0.08
Brain	0.22	0.09	0.04

Table 3. Radioactivity in various tissues at intervals following a single i.v. dose of ^{14}C -HMX (2 mg/Kg) to 6 male and 6 female rats: tissue to plasma ratios.

Tissue	Tissue to plasma radioactivity ratios		
	2 min	24 hr	96 hr
Whole blood	1.5	1.2	1.5
Lung	7.6	1.8	4.3
Liver	2.3	4.6	6.1
Kidney	2.2	4.1	8.1
Skeletal muscle	0.7	1.2	1.7
Brain	0.1	0.5	0.8

METABOLISM

No information on the metabolism of HMX was found by Ryon et al. (1984).

Cameron (1986) determined radioactive HMX and metabolites in urine and feces of rats following a single oral dose of 2 mg/kg ^{14}C -HMX. Urine samples

(0-6 hr, 6-24 hr, 24-48 hr, 48-72 hr, and 72-96 hr) and feces samples (0-24 hr) were pooled for male and female animals separately. The three earliest urine samples for each sex were analyzed by extraction with acetonitrile followed by thin layer chromatography (TLC) on silica gel in dichloromethane:acetonitrile, 80:30 v/v, (each sample was co-chromatographed with unlabelled HMX) and apposition autoradiography. Four significant radioactive components were detected: HMX (at RF 0.51), two minor metabolites called Met 1 and Met 2 (respectively at RF 0.08 and 0.03), and material retained at the origin. The minor metabolites accounted for only 1-2 percent of total radioactivity in both male and female samples, while approximately equal proportions of HMX and origin (polar) material made up the remainder. The proportion of total radioactivity associated with HMX was higher in female samples and tended to decrease with time. At 48 hours, the following amounts, expressed as percent of total administered radioactivity, had been eliminated in urine:

	Male	Female
HMX	24.5	37.8
Met 1	0.2	0.2
Met 2	1.0	1.0
Polar (origin)	28.3	16.7

All the pooled urine samples (through 96 hr) and the pooled feces samples (0-24 hr) were analyzed by high performance liquid chromatography (HPLC). Samples were extracted with acetonitrile and taken up in 90 percent methanol. Reverse-phase HPLC was performed on 4 X 0.5 cm Co-Pell ODS and 25 X 0.8 cm Hypersil ODS columns (mobile phase - 25 percent acetonitrile, detection - u.v. absorbance at 280 nm). Each sample was co-chromatographed with unlabelled HMX. Radioactivity in fractions was detected and quantitated by scintillation counting. The pattern of metabolites was the same for all samples; only the amounts and proportions varied (Table 4). There were two major components - ^{14}C -HMX, at a retention time of 22 minutes, and polar material unresolved from the solvent front. In addition three minor components were detected (A, B, and C). These accounted for less than 2 percent of each urine extract, although one, Met C, accounted for up to 10 percent of the small amount of radioactivity found in the feces. The proportion of radioactivity associated with HMX generally decreased with time post-dose. Total amounts eliminated in 96 hours were as follows:

	Males	Females
HMX	25.1	40.7
Met A	0.4	0.3
Met B	0.4	0.2
Met C	0.7	1.0
Polar material	29.4	20.1

The polar metabolite(s) was essentially unretained in the HPLC system, always chromatographing at or close to the solvent front. Using acetonitrile-water systems with sodium lauryl sulfate, several experimental mobile phases

were tried, without achieving retention of this material. To investigate the possibility that the highly polar substance is a conjugate, urine was treated with an equal volume of HCl for 1 hr at 100C, neutralized, and prepared for HPLC. No real difference, qualitative or quantitative, was detected as a result of the acid hydrolysis.

Table 4. HPLC analysis of urine ^{14}C from rats up to 96 hr after a single i.v. dose of ^{14}C -HMX (2 mg/Kg), results expressed as percent of dose administered (M - male, F - female). HPLC mobile phase - 25% acetonitrile; detection - UV absorbance at 280 nm.

Component		0-6	6-24	24-48	48-72	72-96	0-96 hr
Highly polar,	M	10.8	15.2	2.1	0.8	0.3	29.2
	F	5.4	9.0	2.3	1.0	0.2	17.9
Metabolite A,	M	0.2	0.2	0.0	0.0	0.0	0.4
	F	0.1	0.2	0.0	0.0	0.0	0.3
Metabolite B,	M	0.1	0.2	0.0	0.0	0.0	0.3
	F	0.0	0.2	0.0	0.0	0.0	0.2
Metabolite C,	M	0.3	0.3	0.0	0.0	0.0	0.6
	F	0.2	0.5	0.1	0.0	0.0	0.8
HMX	M	14.0	10.0	0.6	0.1	0.1	24.8
	F	13.0	22.4	2.9	1.0	0.2	39.8
Remainder	M	0.4	0.2	0.0	0.0	0.0	0.6
	F	0.2	0.1	0.0	0.0	0.0	0.3
Unextracted	M	1.0	4.0	0.1	0.0	0.0	5.1
	F	0.3	1.0	0.4	0.1	0.0	1.8
Total	M	26.8	30.1	2.9	1.0	0.4	61.2
	F	19.3	33.6	5.8	2.2	0.5	61.4

Cameron (1986) determined HMX in terminal plasma samples collected at 2 min and 24 hr following i.v. administration to male and female rats. Eluate was collected from the HPLC column at the retention time of authentic ^{14}C -HMX to assess HMX radioactivity as a percent of total plasma radioactivity. At 2 min post-dose, 87 to 98 percent of plasma radioactivity was unchanged HMX, amounting to 0.7 to 1.9 $\mu\text{g}/\text{ml}$, whereas at 24 hr, only 10 to 14 percent of the circulating radioactivity was HMX, corresponding to about 0.02 $\mu\text{g}/\text{ml}$, which is near the limit of sensitivity of the HPLC method used. When twenty-four hr plasma samples were extracted with methanol, in an effort to improve the extractability, it was found that most of the small amount of radioactivity present was associated with the precipitated protein fraction. Thus much of this radioactivity may be strongly bound or incorporated into plasma protein.

Distribution of ^{14}C -HMX and polar metabolite following a single i.v. dose was studied by Cameron (1986). Selected tissues from male and female rats sacrificed at 2 min, 24 hr, and 96 hr post-dose were pooled and extracted with homogenization in methanol and prepared for HPLC analysis. The metabolic profile of each sample, with the exception of the 2 min liver extracts, contained only HMX and/or the polar component. In 2 min liver extracts, a further, minor, component was observed with retention characteristics close to those of HMX. The polar material displayed properties similar to those of the polar component identified in urine samples. Distribution of ^{14}C -HMX and the polar component is presented in Table 5. At 2 min almost all the radioactivity was present as unchanged HMX, except in liver samples, where about 30 percent was present as the polar material. Levels of radioactivity were much lower in later samples, and some samples could not be assessed for this reason. There was in general a much higher proportion of the polar component in later samples, especially in liver tissue.

Table 5. Levels of HMX and polar metabolite in tissues of rats at 2 min, 24 hrs, and 96 hrs following i.v. administration of ^{14}C -HMX (2 mg/Kg).

Sample	Percent as HMX		Percent as polar metabolite	
	Male	Female	Male	Female
2 min				
Liver	62.0	45.7	28.8	39.1
Lung	97.5	97.2	0.3	0.6
Kidney	82.1	88.8	1.1	10.4
Brain	93.8	65.3	1.2	4.9
Skeletal muscle	88.2	88.3	0.8	1.7
24 hr				
Liver	0	0	88.7	55.0
Heart	52.9	*	20.7	*
Kidney	33.1	43.7	34.6	21.4
96 hr				
Liver	*	0	*	63.4

0 - undetectable; * - not reported

No unusual sites of accumulation or retention were identified. The great increase in the proportion of metabolites with time suggests that there is a more rapid removal of ^{14}C -HMX than of the metabolites. It is clear, however, from the fact that $^{14}\text{CO}_2$ is produced, that both ring cleavage and further degradation of ^{14}C -HMX occur. The possibility thus exists that some ^{14}C may have been incorporated into body tissues as natural products.

Because the primary toxic effect of HMX, cardiovascular depression, is characteristic of nitrite toxicity, the cleavage of NO_2 groups may be indicated (Ryon and Pal, 1984). Yet it appears that methemoglobin is not formed (McNamara, 1974). Craig and Done (1985) looked for nitrite in the

stomach contents of mice fed HMX. Traces were found but were not related to the HMX content of the diet.

4. HUMAN HEALTH EFFECTS

Hathaway and Buck (1977) conducted an epidemiologic study of RDX/HMX workers at Holston AAP. The subjects were exposed occupationally to airborne concentrations of RDX up to 1.57 mg/m^3 . HMX was known to be present as well, and of a total of 69 exposed subjects 24 were known to be exposed to both compounds. Testing for a number of hematologic and biochemical parameters failed to reveal any evidence of adverse health effects, in comparison to a control group.

Sunderman (1944) performed patch tests on volunteer workers who were occupationally exposed to RDX, HMX, and other components of the Bachman reaction. Small amounts of dried HMX powder were placed on $7/8" \times 1"$ patches of muslin and applied to the upper arm beneath the axilla for 5 days. The patches were then removed and, after an additional 10 days, were reapplied at the same site for a period of 2 days. In one individual, who kept his patch in place for 8 days, several papules and a small vesicle were observed. No lesions were noted among the four individuals on whom the patches were reapplied after a period of 10 days.

A group of volunteers at the Philadelphia Navy Yard, who had not previously been working with RDX or HMX, was tested in a similar manner, i.e., 5 days on, 10 days off, and a second application in place for 2 days. There were 95 persons: 48 men and 47 women. Of these, 6 men and 6 women gave positive responses to the first application and 5 men and 6 women to the second. With two exceptions, the reactions consisted of minimal erythematous macular and papular lesions and were interpreted as most probably due to primary irritation. In two women, marked erythematous papules or papular eruptions occurred, in one case after the second application and in the other after both first and second applications. In the latter individual there was a history of mild attacks of hay fever. The results in these two persons were interpreted as a true sensitivity response.

In a further experiment, Sunderman (1944) patch-tested four subjects who had previously shown a sensitivity to the fumes from the Bachman reaction. HMX was applied with gauze pads soaked in a saturated acetone solution of the compound. The patches were applied for 5 days; 6 days after removal, fresh pads were applied for 2 days. There were no positive responses before the second application, when one subject developed a simple erythema at the site. The absence of any marked or unusual response on patch testing of individuals who had a history of skin lesions associated with exposure to the Bachman reaction led Sunderman to infer that such lesions were not due to the solid components, including HMX, that they tested.

5. ACUTE TOXIC EFFECTS IN ANIMALS

ORAL EXPOSURE

Kagan et al. (1975) reported acute oral LD₅₀ values for HMX. These were 1500 mg/kg for mice and 300 mg/kg for guinea pigs. Although no LD₅₀ was reported for rats, 3 of ten animals died within 40 days at a dose level of 100 mg/kg/day.

During the recent studies on mammalian toxicology of HMX at Inveresk Research International (Cuthbert, 1985), six groups of Fischer 344 rats (5 males and 5 females per group) were dosed once by gavage with freshly prepared suspensions of HMX in 0.5 percent low-viscosity carboxymethylcellulose (CMC), at a constant dose volume of 20 ml/kg. Mean body weight at dosing was 183 g for males and 131 g for females. Dose levels received by the respective groups were chosen on the basis of a range-finding study in which two animals were used at each of 5 doses. Doses chosen for the main study were equivalent to 0 (control), 2,421, 3,632, 5,447, 8,187, and 12,256 mg dry HMX/kg. Animals were observed twice daily for 14 days, and at death or termination gross post mortem examination was performed.

Mortality was 0/10, 0/10, 4/10, 8/10, and 10/10 in the five experimental groups, respectively. There were no deaths in the control group. Oral LD₅₀s (with 95 percent confidence limits) were calculated by the probit method to be:

males	-	5.51 (5.02-5.93)	g/kg
females	-	6.44 (5.84-7.05)	g/kg
combined	-	6.25 (5.85-6.65)	g/kg

Clinical signs included piloerection and ataxia, and both hyperkinesia and hypokinesia were noted. Post mortem observations included white fluid in the stomach and intestinal tract, pale kidneys, and abnormally pink lungs. There were no clinical signs or post mortem abnormalities in the control group.

A similar study was done by Cuthbert (1985) using mice (B6C3F1, mean weight for males 22 g and for females, 18.5 g). The method of dosing was the same as for the rats (above), with a constant dosing volume of 20 mL/kg. Groups of 5 animals of each sex were given, respectively, 0, 956, 1,626, 2,764, 4,699, and 7,988 mg/kg HMX, dry weight basis. As in the rat experiment, the doses were chosen following a small range-finding experiment. The mice were observed twice daily for 14 days, and at death or termination were subjected to gross post mortem examination.

Mortality was, respectively, in males, 0/5, 4/5, 5/5, 5/5, 5/5, and in females, 0/5, 0/5, 0/5, 5/5, 5/5. Oral LD₅₀ values were calculated as:

Males	-	1.67 (1.44-1.89)	g/kg/day
Females	-	3.24 (2.92-3.57)	g/kg/day
Combined	-	2.30 (2.11-2.50)	g/kg/day

Clinical signs included piloerection, soiled coat, hyperkinesia, hypokinesia, ataxia, sedation, and swollen penis. Post mortem observations included white fluid filling the stomach and intestinal tract, gut contents fluid or gut filled with blood, stomach wall white, and lungs red. There were no clinical signs nor post mortem abnormalities in the control group.

An assessment of the acute oral toxicity of HMX was also performed in New Zealand White rabbits (Cuthbert, 1985). Mean body weight was 2.95 kg for males and 2.82 kg in females. The animals were dosed once by means of a rubber catheter with a freshly prepared suspension of HMX in carboxymethylcellulose at a constant dose volume of 10 mL/kg. One animal of each sex was dosed at each of the following levels: 50, 100, 250, 428.5, 1000, and 2000 mg dry HMX/kg body weight. The rabbits were observed twice daily for 14 days. At death or termination each was subjected to a gross post mortem examination. Mortality was 1/2 in each of the two lowest dose groups and 2/2 in the remaining groups. Because of the small numbers, LD₅₀ was not calculated. Clinical signs included hyperkinesia, hypokinesia, and clonic convulsions, with both miosis and mydriasis being reported. Post mortem observations included irregular reddening on lungs, mottled or pale kidneys, and fluid plus particulate material in the thoracic cavity.

INTRAVENOUS EXPOSURE

The effect of i.v. administration of HMX is circulatory system collapse, with delayed central nervous system disturbances - hyperactivity and convulsions (McNamara et al. 1974, Ryon et al. 1984).

McNamara et al. (1974) injected HMX (10% in DMSO) into the caudal veins of 20-g mice, at dose levels of 15, 25, 30, 35, and 50 mg/kg, using six animals per dose. They were observed for 4 hrs and daily for 30 days. The LD₅₀ was calculated to be 28.9 (25.1-33.3) mg/kg, in comparison to 18.7 mg/kg for RDX in the same experiment. Deaths occurred in 5 to 10 min and were preceded by mild convulsions and labored breathing. Survivors were lethargic for several hours but appeared normal at 24 hr.

Solutions of HMX and RDX (separately), 33 percent wt/vol in DMSO, were given to guinea pigs i.v., two animals per dose (McNamara et al., 1974). The LD₅₀ was calculated to be 28.2 (20-39.8) mg/kg for HMX, compared to 25.1 mg/kg for RDX. Death occurred within five min, preceded by convulsions and prostration. There were no clinical signs in control animals, receiving DMSO alone.

Acute intravenous toxicity of HMX was assessed by Cuthbert (1985) in rats and rabbits. Fischer 344 rats were used in groups of five males and five females per dose level, the doses being chosen following a small range-finding experiment. Animals were dosed once with fresh, filtered DMSO solutions of HMX at a constant concentration of 250 mg/mL. Dose levels were 15, 19.5, 25, 32.5, and 42.5 mg/kg (males) and 30, 45, 67.25, 101.5, and 152.5 mg/kg (females). Control groups received vehicle only. Animals were observed for 14 days, and all were subjected to gross post mortem examination

The intravenous LD₅₀s were calculated by the method of probit analysis to be:

Males - 25.13 (23.04-27.41) mg/kg
Females - 38.08 (32.25-43.91) mg/kg

Clinical signs included hyperkinesia, increased regular breathing, vocalization, clonic convulsions, paralysis of the hind limbs, and coma. Post mortem findings included red foci and dark red patches on all lung lobes. There were neither deaths, clinical signs, nor abnormalities post mortem in the control group.

In the rabbit experiment, a dose-ranging study, Cuthbert et al. (1985) used animals of the New Zealand White strain, weighing on average 3.0 kg (males) or 3.11 kg (females). The test substance was given in fresh, filtered DMSO solution, at varying concentrations and volumes. One male and one female rabbit were used in each of seven dosage groups: 1.0, and 10 mg/kg at a concentration of 50 mg HMX/mL DMSO; and 5, 10, 12.5, 15, and 20 mg/kg at 250 mg HMX/mL DMSO. Controls received DMSO only. The animals were observed for 14 days, and all were subjected to gross post mortem examination. In this, as in the foregoing experiment using rats, deaths occurred almost immediately after dosing. Mortality was 0/2 and 2/2, respectively, in the two pairs dosed with solution at 50 mg HMX/mL and was 0/2, 0/2, 1/2, 2/2, and 2/2, respectively in the five pairs dosed with 250 mg/mL solution. Thus the intravenous LD₅₀ was in the range 10-15 mg/kg. Clinical signs included hyperkinesia, aggression, vocalization, difficult breathing, prostration, immobility, slight epistaxis, dyspnea, and clonic convulsions. No abnormalities were detected post mortem. There were no deaths or clinical signs in the control pair.

A study was performed by McNamara et al. (1974) to assess and define the effects of HMX in various solvent vehicles on physiologic and central nervous system function. Beagle dogs, averaging 11.4 kg were used, with the test substance administered in each of three solvents, DMSO, cyclohexanone, and acetone. Physiologic parameters monitored included EEG (via implanted electrodes), EKG, vital signs, reflexes, clinical signs, and responses to various stimuli.

Six animals were given 40 mg/kg HMX as a 33 percent (wt/vol) solution in DMSO; in two of these the dose was divided into two 20 mg/kg doses 30 min apart. In the four receiving single doses, a severe cardiovascular collapse occurred, with narrow pulse pressure, bradycardia, and respiratory alterations. The EEGs were characterized by high-voltage, low-frequency discharges. Two animals died within 3 min; the remaining two survived the circulatory collapse but died at 14+/-2 hr. The two divided-dose animals also showed severe cardiovascular depression. One died in 1 min, while the other showed epileptic-like EEG and muscle movements, hyperactivity, vomiting, and extreme sensitivity to light and stroke stimuli, and died in about 14 hr. Vehicle controls showed that the results were not explainable by the exposure to DMSO.

Two dogs each received a dose of 20 mg/kg HMX (33 percent in DMSO). In one, hyperreflexia occurred at 2 hr, convulsive seizures and hyperactivity at 5 hr, and recovery at about 5 days. In the second animal, from which the restrainer had been removed, rapid breathing and hyperpnea, cyanosis, retching, salivation, faint pulse, and prostration were observed within the first 23 min. At 3 to 3 1/2 hr, labored breathing, subconvulsive jerking in response to auditory stimuli, and then clonic-tonic convulsions and opisthotonus occurred. Death occurred at 12-17 hr.

Several dogs received HMX in vehicles other than DMSO, the doses being smaller because of the lower solubility. Doses of 2.5 mg/kg and 6.75 mg/kg in acetone were given, the larger producing a blood pressure drop, high-voltage low-frequency EEG discharges, and depressed respiratory rate. A dose of 3.1 mg/kg in cyclohexanone produced cardiovascular collapse, tremors, and a comalike state. All of these animals fully recovered.

DERMAL TOXICITY

A study of the acute dermal toxicity of HMX in rats and rabbits was performed by Cuthbert et al. (1985). Eight Fischer 344 rats of each sex were used, averaging 196 g. The dorsal surface of the trunk was shaved and abraded with a lancet, in such a way as to penetrate the stratum corneum but not the epidermis. Freshly prepared HMX, 600 mg/mL in physiological saline, was applied on a piece of gauze that covered approximately 10 percent of the animal's body (actual measurement of treated area not stated). The dose was equivalent to 4.23 g HMX/kg. The gauze was covered by an impervious material for 24 hr, after which it was removed and any remaining test material wiped off. Rats were observed for 14 days and were weighed at 7 days and at termination. Gross post mortem examinations were performed. There were no deaths and no abnormalities at post mortem in treated or control groups. No abnormalities were noted on histopathological examination of haematoxylin/eosin-stained sections of treated skin. Therefore the percutaneous LD₅₀ is in excess of 4.23 g/kg in these animals.

A similar lack of sensitivity to HMX applied topically was reported in guinea pigs by McNamara et al. (1974). HMX (33 percent wt/vol) was applied to the clipped backs of the animals at doses from 316 to 3000 mg/kg (four animals per dose; size of skin area used not stated). Slight erythema was noted at doses of 1000 mg/kg and above, but only at the highest dose, which consisted of three applications of 1000 mg/kg each, were any non-localized effects noted. These were apprehension and loss of weight.

Cuthbert et al. (1985) also studied the acute dermal toxicity of HMX in New Zealand White rabbits, mean body weight: males 3.13 kg, females 3.08 kg. In this study both abraded and non-abraded skin was treated, and the test article was applied in 1 percent carboxymethylcellulose. Animals were assigned to 8 test groups, each with 4 males and 4 females. Two test groups, abraded and non-abraded, were treated at each of 4 dose levels chosen on the basis of a smaller dose-ranging experiment. The treatment levels were 168, 372, 816, and 1788 mg/kg (size of skin area used not stated).

The percutaneous LD₅₀s and 95 percent confidence intervals in rabbits were calculated to be:

Male, non-abraded	-	634 (532-736) mg/kg
Male, abraded	-	674 (562-785) mg/kg
Female, non-abraded	-	719 (596-842) mg/kg
Female, abraded	-	1337 (415-1759) mg/kg
Combined	-	982 (861-1103) mg/kg

Clinical signs observed at the lower dose levels (168 and 372 mg/kg) included hyperkinesia, hypokinesia, miosis and mydriasis, clonic convulsions, aggressiveness, and stiffness/paralysis of hind limbs. At the highest dose level, breathing difficulty, cyanosis, and prostration were also observed. The clinical signs tended to be delayed in onset, while the convulsions and behavioral effects clearly bespeak a CNS effect.

Histopathological findings at the lower dose levels were:

- Liver - Congestion, eosinophilic cytoplasm, centrilobular necrosis (one case)
- Kidney - Chronic nephropathy
- Lung - Alveolar exudate, alveolar degeneration (one case).
- Spleen - Red pulp depletion (one case)

In addition, at the higher dose levels, white pulp depletion of the spleen was recorded in 10 of the 32 animals. Alveolar hemorrhage was found in 15 of 32, as compared to 1 of 16 in the control groups. Renal congestion was also commonly found at the two higher dose levels. Increased hepatic cellularity was noted in two animals at the highest dose level. The histopathological findings did not identify any specific cause for either the observed clinical signs or the mortality in these animals.

EYE IRRITATION

Eye irritation tests on HMX, both in distilled water and as a dry powder, were performed by Cuthbert et al. (1985). To test the material in the wet form, 0.1 mL of the preparation, 60 percent HMX in water, was instilled into the right eye of each of three male and three female New Zealand White rabbits, weighing 2.5-3.0 kg. Left eyes served as controls. Ocular reaction was scored at 1 hr and 24 hr and at 2, 3, and 7 days, using an FDA-recommended scoring system. No positive responses of the cornea or iris were noted. Slight redness of the conjunctiva, 2/6 treated eyes at 1 hr, 1/6 at 7 days, was observed; 5/6 eyes were normal at 24 hr. When the same experimental protocol was followed with 500 mg of dried powder, there were again no corneal or iridial responses. All animals showed slight redness of the conjunctiva at 1 hr, and 2/6 animals at 24 hr. Two rabbits showed mild conjunctival chemosis. All eyes were normal at 2 days. These results indicate that HMX, whether wet or as a powder, is not an eye irritant.

6. SUBCHRONIC AND CHRONIC TOXIC EFFECTS

ORAL TOXICITY

To study the subacute toxicity of HMX and to identify suitable dose levels for planned chronic (13-week) feeding studies, 14-day studies in rats and mice were carried out at Inveresk Research International (Greenough and McDonald, 1985a,b).

Fischer 344 rats were fed diets delivering nominal doses of 0, 333, 1000, 3000, and 9000 mg HMX/kg/day, six animals per sex per dose (Greenough and McDonald, 1985a). There were 13 deaths, 11 of which were in the highest dose group. There were marked reductions in food consumption and weight gain, which tended to be dose-related. The highest-dose male group and the three high-dose female groups weighed less at the end of the two-week dosing period than at the start. Clinical signs included emaciation, hunched posture, hyperkinesia, and piloerection. One convulsive episode occurred among the high-dose females. Histopathological examination, performed on all control and highest-dose animals, revealed centrilobular degeneration in livers of male animals and hepatocyte hyperplasia and eosinophilia in females. In females receiving the highest dose, there was depletion of lymphocytes in the thymus and spleen and depletion of red and white pulp in the spleen. These females also had smaller than normal spleens and enlarged adrenals. Absolute liver and kidney weights were reduced in all treated groups. Relative liver weights were reduced in males, while relative kidney weights were increased for both sexes. It is likely that much of the drop in food consumption and weight gain was due to unpalatability of the feed, which also made the actual achieved doses somewhat erratic and the organ weight data difficult to interpret. The histopathological findings indicate the liver to be a target organ for HMX effects.

In the 14-day feeding study in B6C3F1 mice (Greenough and McDonald, 1985b), males were fed HMX in the diets; six animals were used at each indicated dose level. Mortality was distributed as follows:

Nominal dose	- Males -					- Females -				
mg/kg/day	0	100	300	900	2700	0	320	800	2000	5000
% Mortality	0	0	83	100	100	0	0	33	67	100

Clinical signs noted in treated animals were similar to those in the rat study: piloerection, hunched posture, emaciation, and hyperkinesia. There were two convulsions observed in the 300 mg/kg/day group. Adverse effects on food consumption and weight gain were seen but were much less pronounced than in the rat study. Absolute organ weights were similar in treated and control groups. Histopathological examination of the premature decedents in the treated groups showed dose-related increase in hepatocellular hyperplasia and eosinophilia, splenic red and white pulp cellular depletion, and thymic cellular depletion. There were no sex differences in the histopathological findings.

The two-week feeding studies were followed by 13-week studies in both rats and mice. In the rat study (Everett et al., 1985), five groups of 20 male and 20 female Fischer 344 rats were given diets calculated to provide the following dose levels: for males, 50, 150, 450, 1350, and 4000 mg/kg/day and for females, 50, 115, 270, 620, and 1500 mg/kg/day.

There were only three premature decedents, including one control animal and one receiving only 150 mg/kg/day. No clinical signs were noted that could be attributed to HMX feeding. There was a dose-related reduction in mean body weight, attributed in part to low palatability of the dosed feed. Both food consumption and weight gain improved in the latter weeks of the test.

Hematological examination of high-dose males (4000 mg/kg/day) and females (1500 mg/kg/day) in the fifth and twelfth weeks revealed statistically significant decreases in hemoglobin and hematocrit (P less than 0.001) in both sexes and increased methemoglobin (significant in males, P less than 0.05). While apparently due to HMX exposure, these changes were quite small and not likely to be of much health significance. Neutrophils and total WBC were significantly increased in females at 12 weeks, though the relation of this to HMX exposure is uncertain.

Clinical chemistry results from highest-dose groups in weeks 5 and 12 showed considerable difference between the sexes (Table 6). Alkaline phosphatase (AP) levels were increased in males (P less than 0.001 in week 12) but only to a much lesser extent in females. Males also showed reduced alanine aminotransferase (P less than 0.01 in week 5). Blood urea N (BUN) and total protein were increased in high-dose females (P less than 0.05 in week 5, 0.001 in week 12). Albumin was significantly increased in both sexes.

Urinalyses were also carried out in the fifth and twelfth weeks, on samples from the highest-dose group of each sex (Table 7). In the females there was a reduced pH and specific gravity with a corresponding increase in urinary volume. These changes were essentially lacking in males. In the week 12 samples from both sexes, fern-like crystals were noted.

When significant body weight effects are seen, it is difficult to interpret organ weight differences, either absolute or relative. Thus the meaning of the minor changes in testis, adrenal, spleen, and ovary weights is questionable.

Examination of all major organs revealed only two types of histopathological lesion that were dose-related and attributable to HMX dosing. Toxic change in the liver was characterized by enlarged cells, mainly in centrilobular areas, with large, pale nuclei and dark, granular, eosinophilic cytoplasm. Sometimes there were dilation of sinusoids and small foci of necrosis. This liver pathology was far more prevalent in males - almost all males receiving 450 mg/kg/day or above - than in females (only one animal, receiving 270 mg/kg/day). Toxic change in the kidney was characterized by focal atrophy and dilation of the tubules. This condition was prevalent in females, while in males it was no more frequent than in control animals.

Table 6. 13-Week dietary toxicity study in Fischer 344 rats (Everett et al. 1985): clinical chemistry results from control and high-dose animals (males - 4000 mg/kg/day, females - 1500 mg/kg/day). All values based on 10 individuals.

Test	week	Males				Females			
		Control mean	S.D.	Treated mean	S.D.	Control mean	S.D.	Treated mean	S.D.
BUN (mmol/L)	5	6.4	0.8	6.5	1.0	6.3	0.8	7.2	1.0*
	12	6.4	0.4	6.7	0.9	6.5	0.8	7.7	0.5***
Glu (mmol/L)	5	8.14	0.78	7.72	0.71	7.97	0.58	7.98	0.61
	12	8.32	1.36	7.73	0.52	8.25	1.19	7.86	0.96
AST (IU/L)	5	83	5	78	7	77	8	82	5
	12	122	27	104	21	90	23	84	20
ALT (IU/L)	5	72	7	63	6**	56	7	59	5
	12	65	19	48	23	62	34	57	11
AP (IU/L)	5	712	85	840	64**	635	52	676	73
	12	213	18	415	102***	467	80	553	61*
LDH (IU/L)	5	357	85	417	164	261	46	254	60
	12	278	65	286	119	470	126	417	174
Na, mmol/L	5	146	2	147	3	145	2	146	2
	12	145	3	147	4	144	1	146	2**
K, mmol/L	5	4.2	0.4	4.1	0.3	4.3	0.3	4.3	0.3
	12	4.3	0.3	4.0	0.3*	4.6	0.4	4.8	0.6
Tot.prot., g/L	5	62	2	63	2	59	2	62	2*
	12	70	2	69	2	61	2	64	1***
Alb., g/L	5	37	1	39	1***	34	2	36	1*
	12	41	1	42	1*	37	1	40	1***

* Statistically different from control, P less than 0.05; **, P less than 0.01; ***, P less than 0.001

It is probable that the increase in AP was associated with the toxic liver lesions, both being more prevalent in males than females. Similarly the increase in BUN may be associated with renal damage, both far more prevalent in female rats. Increases in albumin and total protein may also be linked to kidney and liver damage. The increased urinary volume, with lowered specific gravity, and decreased urinary pH found in high-dose females might be anticipated in view of the renal histopathology present in these animals. Since neither of these effects was present in males, a difference between the sexes is suggested in their response to HMX. Different target organs in males and females could reflect a difference in the metabolism of HMX. There was no evidence of toxic liver change in male rats at 50 mg/kg/day or of renal effects in female rats at 115 mg/kg/day or less.

In the 13-week feeding studies in B6C3F1 mice (Everett and Maddock, 1985), six groups of 20 male and 20 female animals were fed diets calculated to deliver the following dose levels: for males, 0, 5, 12, 30, 75, and 200 mg/kg/day and for females, 0, 10, 30, 90, 250, and 750 mg/kg/day.

Table 7. 13-Week dietary toxicity study in Fischer 344 rats: urinalysis results from control and high-dose animals (males - 4000 mg/kg/day, females - 1500 mg/kg/day; Everett et al., 1985).

Test	week	Males				Females			
		Control		Treated		Control*		Treated	
		mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
pH	5	8.8	0.3	8.6	0.6	8.7	0.4	7.3	1.4
	12	8.7	0.7	8.1	0.7	8.1	1.1	6.6	0.5
Spec.Grav.	5	1.054	0.024	1.060	0.015	1.061	0.016	1.038	0.023
	12	1.049	0.016	1.049	0.013	1.051	0.018	1.019	0.004
Volume (mL)	5	0.8	0.3	0.7	0.3	0.5	0.0	1.5	0.9
	12	1.0	0.5	1.0	0.4	0.6	0.2	2.7	1.1

* Female control derived from 9 animals (5 weeks), 8 at 12 weeks; all other values based on 10 individuals.

Statistically significant mortality occurred only in the 200 mg/kg/day males (13/20), the 250 mg/kg/day females (12/20), and the 750 mg/kg/day females (20/20). However, despite the substantial mortality levels, little evidence of toxic change was found in the rather thorough investigation carried out.

Mice in the two highest dose groups in each sex appeared slightly more active than other mice during the final two weeks, and females receiving the highest dose were more excitable and jumpy from weeks 4 through 7. No other clinical signs were noted that could be attributed to the test substance. Small statistically significant increases in body weight gain, when compared to controls, were observed in male mice receiving 75 mg/kg/day and in females receiving 30 or 250 mg/kg/day during portions of the 13-week period. Food consumption, however, was reduced in 75 mg/kg/day males (14 percent), 250 mg/kg/day males (19 percent), and 750 mg/kg/day females (9 percent).

Hematology studies revealed no dose-related trends in male mice. Statistically significant differences from control values were occasionally encountered, e.g., increased packed cell volume in the 30 and 75 mg/kg/day groups; but these were felt by the authors to be due to data structure and not of biological significance. A slight reduction in hemoglobin and slight increases in WBC and lymphocyte counts were seen in treated females at the higher dose levels. Heinz body preparations were negative.

Clinical chemistry determinations were performed on blood samples obtained at termination. In males receiving 200 mg/kg/day, levels of glucose and alanine aminotransferase were slightly depressed, as was the albumin/globulin ratio (A-GR). Alkaline phosphatase levels were slightly

reduced in both sexes in the 200-250 mg/kg/day dose range, this being statistically significant only in the females. In male mice, BUN and A-GR showed slight downward trends and statistically significant reductions below control values. This was highly significant for both BUN and albumin in the 75 mg/kg/day group groups, though all values fell within the normal range. In female mice receiving 30 mg HMX/kg/day, a statistically significant elevation in albumin, again within the normal range, was detected.

Table 8. Toxic liver and kidney changes following 13-week dietary administration of HMX to Fischer 344 rats.

Dose mg/kg/day	No. of animals	No. with liver effects	No. with kidney effects
Males			
0	20	0	3
50	20	0	0
150	19	2	0
450	20	20	0
1350	20	18	2
4000	20	20	1
Females			
0	20	0	1
50	20	0	0
115	20	0	1
270	20	1	4
620	20	0	13
1500	19	0	10

Urinalysis was performed during week 13. In males there was a downward trend in both volume and specific gravity of the urine, and this was evidenced in both the 75 and 200 mg/kg/day groups. In the 200 mg/kg/day males, urine was more acidic (pH 6.9 as compared to 8.0). A slight reduction in volume was seen in the 90 and 250 mg/kg/day female groups. There were no surviving mice available for urinalysis in the 750 mg/kg/day female group.

A slight increase in absolute brain weight ($P < 0.001$) was observed in males receiving 200 mg/kg/day. This was observed, without statistical significance, at the 75 mg/kg/day treatment level. Other organ weight data from males (heart, kidneys, liver, lung, spleen, testes) showed no dose-related trends, although there was a statistically significant ($P < 0.01$) reduction in relative testis weight at the 30 mg/kg/day level. Absolute brain weight was increased also in females of the 250 mg/kg/day group ($P < 0.01$), while absolute and relative spleen weight was reduced. Study of the organ weights of female mice receiving 750 mg/kg/day could not readily be done because most were found dead, allowing autolytic processes to commence before autopsy.

Gross pathology and histopathology yielded no dose-related findings. Three animals, in low or intermediate treatment groups, had cysts in the brain, and one in the spinal cord; but these were not thought to be related to HMX exposure.

None of the clinical or laboratory findings was sufficient to offer a mechanism for the mortality observed in the mice receiving doses of 200 mg HMX/kg/day or greater.

CUTANEOUS EFFECTS

McNamara et al. (1974) applied HMX in solvents to the clipped backs of rabbits. One-ml and 0.1 ml volumes of HMX, 33 percent in DMSO, 2.5 percent in cyclohexanone, or 2.0 percent in acetone, were applied daily, 5 days/week for 4 weeks, to six animals for each solution. There was no gross or microscopic evidence of cutaneous irritation except for mild desquamation occurring at 7 days. The incidence of dermatitis, determined at necropsy, did not differ from that in animals serving as solvent controls. There were three deaths in the 1.0 ml DMSO solution group, although hematology, blood chemistry, and pathology studies produced no positive findings.

In a similar experiment in dogs (McNamara et al. 1974), 10 ml of each solution (as above) was applied to the clipped backs of the animals daily, 5 days/week for 4 weeks. Four animals received the DMSO solution (289 mg/kg per application) and two were used with each of the other solvents. Blood pressure, heart and respiratory rates, EKG, and EEG, as well as motor and neural responses to a battery of stimuli, were recorded weekly. No consistent increase or decrease in any of the physiologic parameters was noted. There was no consistent enhancement or blockage detected in any of the responses monitored. During weeks 2 and 3 there was slight erythema and desquamation of the skin on the back, but these were present also in the single DMSO control animal. The absence of positive effects indicates a failure of the test substance to penetrate the skin of these animals.

A primary skin irritation test was performed in New Zealand White rabbits by Cuthbert et al. (1985). Test material (HMX in physiological saline, 60 percent wt/wt) and control material (sodium laurel sulfate, 10 percent) were applied in 0.5 ml amounts to the clipped and abraded back and flanks of six rabbits, three of each sex, weighing 2.5 to 3.0 kg. The filter paper patches (25 cm²) were covered for 24 hr, then removed and the sites wiped. Sites were scored at 24 and 72 hr and evaluated according to the FDA scoring system. A primary skin irritation score of 0.67 (mild irritant), based on the distribution and severity of edema and erythema, was calculated for HMX.

SENSITIZATION POTENTIAL

Sensitization studies of HMX were carried out by McNamara et al. (1974), in guinea pigs, using both intradermal and topical challenge routes. In the

initial, sensitizing, phase, animals were exposed to the test compound 3 days/week for 3 weeks. Topical applications consisted of 0.5 ml and intradermal injections of 0.05 ml of HMX solution (2.0 percent in acetone, 2.5 percent in cyclohexanone, or 3.3 percent in DMSO, the dose levels having been chosen to prevent mortality from HMX toxicity). Following a two-week rest period, each animal was challenged with both a topical and an intradermal dose, one on each thigh at different times. The intradermal challenge doses consisted of dilutions, in saline, of 1:1 (v/v) solvent-saline mixtures while the topical challenge doses consisted of dilutions of the same mixtures in PEG 200. No evidence of sensitization was found from any of the combinations of exposures involving either HMX or solvent controls.

The allergenic potential of HMX was tested also by Cuthbert et al. (1985) using the Magnusson-Kligman maximization test with Freund's complete adjuvant in female Dunkin-Hartley guinea pigs. Simultaneous tests were run on p-phenylenediamine and sodium laurel sulfate as positive and negative controls, respectively. Twenty-five animals were used with each compound. The induction phase consisted of a course of intradermal injections (with a total of 0.15 ml of 6.67 percent HMX in distilled water) followed 6 days later by a topical dressing of 60 percent HMX in distilled water.

The challenge procedure also consisted of a topical application and was carried out 21 days after the induction phase. The test material was applied to the shaved flanks of the animals, at a concentration determined to be non-irritating (60 percent w/v, in paraffin oil) with a 2 X 2 cm patch of filter paper occluded and held in place for 24 hours. In the group of 25 animals challenged with HMX, no positive responses were elicited. Thus there was no indication that HMX is a sensitizer.

MUTAGENESIS

Stilwell et al. (1977) tested the mutagenicity of pilot treatment plant wastewater, both before and after treatment, at the Holston AAP, TN. They used the Ames histidine reversion assay in Salmonella, with five strains of the organism, both with and without metabolic activation. Results were negative or attributed to chance variation. Levels of HMX and RDX ranged from 0.1 to 5.0 ppm before treatment and from 0.05 to 0.7 ppm after treatment.

Negative results, again with five tester strains with and without activation, were obtained for HMX by Whong (1980) and Whong et al. (1980). Both spot test and plate incorporation methods were employed. Epler (1985), as reported in Parmer et al. (1985), also failed to find mutagenic activity in the Ames test, as did Simmon et al. (1977; Cotruvo et al. 1977), who used five tester strains, with and without activation, at 0.25 ml saturated HMX solution per plate.

In the Simmon study, HMX, with and without metabolic activation, was also tested in the Saccharomyces mitotic recombination assay. Mitotic recombination at the adenine locus, an indication of mutagenicity, was not observed in either study.

Amounts of the compound tested, in both the Ames and Saccharomyces systems, were small: 1.0 ml of a saturated aqueous solution per plate was used in the Simmon et al. study, while Cotruvo et al. used a solution of 0.3 ppm HMX. Thus, mutagenicity of the test substance at higher concentrations is not ruled out by these findings. McCormick et al. (1984a) tested concentrations of HMX up to 5000 μ g/plate using the Ames reversion assay, with solution in DMSO used to achieve the higher concentrations. Five tester strains were used with and without metabolic activation. The tests were run in triplicate, and a two- to threefold increase in back mutations was considered a positive test. The results were negative.

CARCINOGENICITY

No studies were found that addressed the possible carcinogenic effects of HMX.

TERATOGENICITY AND REPRODUCTIVE EFFECTS

No studies were found that addressed the possible teratogenic or reproductive effects of HMX.

7. ENVIRONMENTAL FATE

MIGRATION

Information on translocation of HMX within the environment is limited. Stidham et al. (1979) reported levels of 67 $\mu\text{g/L}$ one mile downstream of the last plant effluent at Holston AAP and attributed this in part to incomplete mixing of river water and effluent. Up to 200 $\mu\text{g/kg}$ was found in sediment samples from selected stations in the Holston River by Sullivan et al. (1977a).

A computer model simulation of the translocation and persistence of HMX was developed by Spanggord et al. (1983) for the Holston River below Holston AAP. Physical and biological degradation and partition to sediment were included, as well as dilution and solubility considerations. It was projected that HMX loads in the receiving river would persist for great distances below the source. Thus if a HMX discharge of 450 ppb had been reduced, largely by mixing and dilution, to 0.3 ppb 20 km downstream, 0.2 ppb would be expected at 125 km downstream.

The movement of HMX through soils was investigated by Greene et al. (1985). A simulated 'pink water' containing TNT, 2,4-DNT, RDX, and HMX (HMX at 4 mg/L) was continuously applied, at flow rates of 40 and 100 mL/day, with and without carbon supplementation (glucose), to a series of soil columns, 40 cm in height and 7 cm in diameter, filled with garden soil. All columns except a sterilized control were inoculated with activated sludge organisms. Column effluent (leachate) was monitored for explosives and transformation products. Rapid breakthrough of HMX and RDX, but not TNT, was observed at both flow rates, with and without glucose, suggesting that the nitramine explosives would easily travel downward in at least some soils and contaminate groundwater.

PHYSICAL DEGRADATION

The primary mechanism responsible for degradation of HMX in aqueous solution is photolysis (Ryon et al. 1984). Spanggord et al. (1982) estimated the rate constant and half-life for HMX photolysis in outdoor sunlight experiments using 0.5 ppm solutions in pure water and filtered Holston River water. The photolysis followed first-order kinetics and was little influenced by humic substances or other chemicals from the waste stream. A half-life of 4 to 5 days was estimated for HMX in the river.

Photolysis rate constants for HMX in pure water, Holston River water and Louisiana AAP treatment lagoon water were determined by Spanggord et al. (1983). Rate constants for HMX were approximately one-third those for RDX, and both were greatly reduced in lagoon water owing to the presence of 'pink water' compounds from TNT operations. The rate constant for photolysis of HMX in the Holston River in late spring was calculated to be 0.166 day^{-1} , with no significant difference according to location upstream or downstream of the

Holston AAP. Using a computer program, Spanggord et al. (1983) calculated photolysis rate constants for depths of 0 to 300 cm below river surface and for the four seasons. At depths of 150 to 200 cm, photolysis rate constants were reduced by about one order of magnitude. An average half-life of 17 days (150 cm depth) was suggested for HMX photolysis in the Holston River and 7900 days in the Louisiana AAP lagoon. Nitrate, nitrite, and formaldehyde, but no N-nitroso derivatives, were observed as transformation products.

Volatilization will not be an important fate process for HMX in the environment. The Henry's law constant was calculated for HMX by Spanggord et al. (1982) to be no more than 0.15 torr M^{-1} . A volatilization rate constant of $2.4 \text{ to } 7.2 \times 10^{-4} \text{ d}^{-1}$ was estimated for a 200 cm-deep body of water. This corresponds to a half-life of 1000 to 3000 days. More recently, Burrows et al. (1989) have calculated the Henry's law constant for HMX, from its vapor pressure, molecular weight, and solubility, to be $2.60 \times 10^{-15} \text{ atm-m}^3/\text{M}$ at 25°C . This is equal to $1.98 \times 10^{-9} \text{ torr M}^{-1}$. Volatilization from a body of water is thus expected to be negligible.

Spanggord et al. (1982) also studied the possible role of chemical reduction of HMX in anaerobic sediments. Reduction of $1.0 \times 10^{-5} \text{ M}$ HMX by ferrous sulfate, reduced hematin, dithionite ion, and HS^- ion was studied. Dithionite ion was the only effective reducing agent tested (30 percent reduction after 7 days at pH 11), and this ion was not deemed an environmentally relevant reductant. It was concluded that chemical reduction in anaerobic environments (sediments) is not likely to be an important process for HMX. Because of the highly oxidized state of the N- NO_2 groups, oxidation was also deemed unlikely to prove an important process.

Hydrolysis of HMX under environmental conditions is expected to be unimportant (Spanggord et al. 1982). It was reported by Hoffsommer et al. (1977) and by Sikka et al. (1980) that the alkaline hydrolysis of RDX follows second-order kinetics and is very slow below 30°C . Under the same conditions, the hydrolysis rate constants for HMX (Spanggord et al. 1982) are more than two orders of magnitude smaller than for RDX. At pH 8 and 15°C , the half-lives of RDX and HMX in aqueous acetone are 22 days and 21 years, respectively.

BIODEGRADATION

Studies on the biotransformation of HMX were performed by Spanggord et al. (1982). Degradation of HMX under aerobic conditions in the laboratory was performed with freshly obtained Holston River water collected downstream of the HMX waste line at Holston AAP. Initial HMX concentration was 4 ppm, and experiments were conducted at 20° to 25°C in the dark, at a pH of 7-8, in river water, in river water with added sediment collected from the HMX waste line (1 percent), and in river water with added nutrient (50 ppm sterile yeast extract or 100 ppm Difco Brain-Heart Infusion). Initial bacterial populations in the flasks were about $2.5 \times 10^5/\text{ml}$. Without added nutrient, there was no detectable decrease in HMX, as determined by HPLC, during 15 weeks of incubation; but with added yeast extract rapid transformation occurred,

resulting in less than 0.1 ppm HMX remaining after three days. Four metabolites, the mono-, di-, tri-, and tetranitroso derivatives of HMX, were detected by HPLC. Attempts to establish enrichment cultures were not successful; nor were efforts to culture the responsible organisms; and transformation of HMX was not observed in river water that had been stored prior to use. Nevertheless the results indicate that biotransformation of HMX may be occurring in wastewaters and receiving streams.

In the same report, Spanggord et al. (1982), determined biotransformation of HMX under anaerobic conditions. Flasks as described above were bubbled with nitrogen gas and sealed. Degradation was not noted after 15 weeks in river water alone. In the presence of the 1 percent sediment supplement, slow disappearance of the test compound took place: a decline from 4 ppm to 2 ppm in 50 days, and to less than 0.2 ppm in 91 days. The process of degradation showed acceleration with time, suggesting growth of the responsible microorganisms. In the presence of the yeast extract supplement, rapid loss of HMX was seen (less than 0.1 ppm remaining at three days), with one HPLC-identifiable metabolite formed. A continuously transferable mixed culture of the anaerobic organisms was obtained that degraded HMX in three days in the presence of added yeast extract. Spanggord et al. (1982, 1983) reported the formation of the nitroso derivatives and of 1,1-dimethylhydrazine.

McCormick et al. (1984a) inoculated anaerobic nutrient broth cultures containing 50 ppm HMX with anaerobic sewage sludge. The concentration of explosive declined by over 60 percent in five days, then leveled off. Products identified were mononitroso- and dinitroso-HMX, as well as methanol.

BIOSORPTION AND BIOCONCENTRATION

Biosorption studies of HMX with mixtures of several species of gram-negative and gram-positive bacteria were reported by Spanggord et al. (1982). They calculated an average biosorption coefficient of 63, defined as $\mu\text{g HMX}$ per dry weight of cells divided by $\mu\text{g HMX}$ per ml supernatant. This suggests that biosorption would not be an important factor in the environment.

Studies of bioconcentration of HMX were not found. This was determined to be of little significance for RDX (Bentley et al., 1977). The bioconcentration factor for HMX was estimated by Burrows et al. (1989) from the octanol-water partition coefficient. The results, 0.49 for fish and 0.00047 for beef fat/feed, indicate no bioconcentration.

8. ANALYTICAL METHODS

Several methods have been developed for the detection and quantitative analysis of HMX in the presence of related compounds. These include volumetric methods, thin-layer chromatography (TLC), high pressure liquid chromatography (HPLC), and single-sweep polarography. Gas chromatographic methods are severely hampered by the thermal instability and low vapor pressure of HMX, so that HPLC methods seem to be the most widely employed for environmental samples.

Volumetric methods, based on titration with iron salts or sodium methoxide, have been used for bulk munitions, but have not been modified to detect the low levels occurring in environmental samples (Sullivan et al. 1977a).

Detection and quantitation by single-sweep polarography (Whitnack 1976), which relies on the different and reproducible peak potentials of various molecular species, has afforded sensitivity to 0.05 mg/L in water.

Thin-layer chromatography on silica gel has been used to detect HMX in water samples (Leach and Hash 1972, Stilwell et al. 1977) and in both water and sediment samples by Sullivan et al. (1977). A sensitivity of about 1.0 mg/L was reported in both studies. Sullivan et al. (1977a) used ethyl acetate as the carrier solvent and absorptiometric detection by ultraviolet light (254 nm).

HPLC is probably the best method for routine monitoring of low levels of HMX in aquatic systems (Sullivan et al. 1979) and tends to afford higher sensitivity than TLC. Stilwell et al. (1977) detected 0.1 mg HMX/L with a precision of 10 percent (lower limit of detection 0.05 mg/L). Brueggemann (1983) adapted HPLC methods to Holston AAP wastewater containing HMX, RDX, TNT, and related compounds. After concentration of the sample by passage through a SEP-PAK[®] C₁₈ cartridge, the explosives were separated on a reverse phase C₁₈ column. The injection volume was 100 μ L. The mobile phase consisted of methanol and water with a linear gradient elution program (increasing methanol concentration), at a flow rate of 1.7 ml/min at 500 psi. HMX in the effluent was determined by absorption at 240 nm. Recovery of HMX was 76 percent, and sensitivity was 100 ng in 100 μ L on column (1.0 ppm).

A reverse-phase HPLC system was used for routine analysis of HMX in water and sediment samples by Spanggord et al. (1982, 1983). HMX, RDX, and other explosive compounds and metabolites were separated by direct injection of water samples or of ethyl acetate extracts of sediment samples into a Waters radial compression module with a C₁₈ RCM cartridge. A linear gradient of water and methanol/acetonitrile was utilized, with detection at 254 nm. The detection limit for aqueous injection was 0.1 mg/L, which could be lowered through a concentration step. For sediment samples, without cleanup, the limit was 0.5 μ g/g. Spanggord et al. (1982) also employed a normal phase HPLC system with detection by a nitrosyl-specific thermal energy analyzer. The detection limit was 8 ng of HMX injected. HPLC methods have been adapted

by McCormick et al. (1984a, 1984b) to the detection of HMX and metabolites in extracts from microbiological culture media used in biodegradation studies; by Henderson (1985a) to extracts from rodent diet and dosing formulations; by Brueggemann (1986) to extracts from deactivation furnace ash; by Jenkins et al. (1984) to wastewater from nitramine manufacture and from load, assembly, and pack (LAP) operations; by Burrows and Brueggemann (1984) to DMSO process effluent; and by Henderson (1985b) to plasma obtained during rodent toxicity studies. In the latter case, the limit of detection, with good accuracy and precision, was 20 ng/mL.

In summary it is apparent that sensitive analytical techniques are available for the separation and quantitation of HMX and associated compounds in industrial, environmental, and biological materials.

9. TREATMENT METHODS

Waste HMX occurs as solid explosive formulations and in wastewater from RDX and HMX manufacturing and from LAP operations. In the last case, TNT and related substances are usually present, and the designation 'pink water' is used. Treatment methods in use or under consideration for the destruction of explosive compounds, including nitramines, prior to environmental release are discussed by Pal and Ryon (1986).

For explosive-laden solid materials, both landfilling and open burning are still practiced at AAP's, but, with the development of new methods and equipment, incineration is becoming the preferred method. Maybury (1982, cited in Pal and Ryon, 1986) states that 80 percent of waste propellants and explosives are now incinerated. At Holston AAP, which manufactures all of the RDX and HMX made in the U.S., solid explosive-laden waste is currently open-air burned in clay-lined pits. There are several types of incinerator equipment, all requiring a fuel source such as gas or oil, in use or under development:

1. In the rotary kiln incinerator, a type of which was installed at Radford AAP in 1978, waste propellant in slurry form is burned by a continuous process at 1000°C in refractory-lined cylinders rotating at a slow speed. Both capital and maintenance costs are relatively high.

2. In the fluidized bed incinerator, studied at Picatinny Arsenal, Dover, NJ, air is forced upward through a bed of aluminum oxide/nickel oxide. As in (1), the propellant is delivered in slurry form.

3. In pyrolytic incineration processes, material to be disposed of is conveyed into the heated zone of a furnace, where both pyrolysis and combustion occur. In a second step, heat is recovered. The system has advantages in the treatment of wet solids and sludges.

4. In the SITPA (Simplified Incinerator Technology for Pollution Abatement) system, waste materials are fed in dry form (making the operation somewhat more hazardous) and burned in an unlined rotary kiln.

Current methods for treatment of HMX-laden waters result in only partial removal of this compound. Holston AAP has, since 1983, used settling, earth filters, and biological treatment towers; here release to the environment, including groundwater, has been a problem. In pilot studies of this system, Hash (1977, cited in Pal and Ryon, 1986), reported HMX removals of 5.4 percent at a flow of 0.8 gpm, and 44.7 percent at a flow of 1.2 gpm. Sludge and contaminated filter material were disposed of by landfilling (Pal and Ryon 1986). Heffinger and Pregun (1985), in bench-scale experiments, evaluated the capability of the Radford AAP rotating biological contactor to remove HMX and

RDX. The results were disappointing.

Pink water, which comes from LAP operations involving TNT and as a condensate from red water disposal operations, and which typically contains both RDX and HMX, is currently treated by passage over granular activated carbon (GAC) columns (Ryon et al. 1984). While this treatment is capable of reducing total nitrocompounds from 140 mg/L to less than 0.3 mg/L, removal of HMX is poor; there is a tendency for HMX and RDX to be displaced from the column by TNT (Burrows, 1984). GAC is not currently in use for treatment of wastewater from the RDX/HMX lines at Holston AAP.

HMX is biodegradable in anaerobic sewage sludge, but complete elimination was not achieved after several weeks of incubation (McCormick et al. 1984b). Two intermediates were identified: the mononitroso and dinitroso derivatives of HMX.

Several forms of tertiary treatment have been shown to be potentially capable of removing HMX and other nitramines from aqueous solutions in bench-scale experiments at Holston AAP. It was shown by Kobylinski and Burrows (1984) that HMX and other nitramines can be reduced to below the detection limit by corona oxidation (the Innova process), an electrolytic process in the presence of graphite fiber particles. This was achieved by two hours of treatment, with the munitions tested both singly and in mixtures. Ultraviolet irradiation was also effective (Burrows, 1983), especially in combination with hydrogen peroxide (Andrews and Osmon, 1976; NWSC, 1985). In combination with 0.01 percent hydrogen peroxide, HMX in pure solutions was degraded with a half-life of only 11.6 minutes (Noss et al. 1984). These processes require further investigation, under realistic conditions and better definition of the energy requirements and cost-effectiveness.

Wastewaters containing HMX and RDX may be successfully treated in continuous culture biological treatment systems, if the systems are operated under anaerobic conditions (McCormick et al., 1984; Heffinger and Pregun, 1985).

10. CRITERION FORMULATION

EXISTING CRITERIA AND STANDARDS

No existing criteria or standards were found for HMX. These have not been established because of lack of data (Pal and Ryon, 1986).

AQUATIC CRITERIA DEVELOPMENT

A brief description of the methodology proposed by the US Environmental Protection Agency for the estimation of water quality criteria for the protection of aquatic life and its uses is presented in Appendix A. Aquatic criteria, as proposed by USEPA, consist of two values, a Criterion Maximum Concentration (CMC) and a Criterion Continuous Concentration (CCC) (Stephan et al., 1985). The CMC is equal to one-half the Final Acute Value, while the CCC is equal to the lowest of the Final Chronic Value, the Final Plant Value, and the Final Residue Value.

Data available for calculating a Final Acute Value for HMX do not meet the requirements specified by the USEPA guidelines (Stephan et al., 1985). Static acute toxicity tests were performed by Bentley et al. (1977) on four freshwater invertebrates, representing four diverse families, and on four species of fish. The list of organisms tested meets most, but not all, of the criteria specified in Stephan et al. (1985). In no case can a mean acute value be derived. This is due in part to low toxicity, the acute value being higher than the solubility limit of the test substance (about 3.3 mg/L). Thus it is unlikely that the Final Acute Value would be less than 3.3 mg/L. Because the studies reported did not include chronic or flow-through testing, a Final Chronic Value cannot at this time be determined.

Bentley et al. (1977) also studied the toxicity of HMX to four diverse species of algae. No deleterious effects were detected in this study, with nominal concentrations ranging up to 32 mg/L, which is well above the solubility limit and therefore well above the actual concentrations achieved. Small statistically significant effects on cell density and chlorophyll a were later found in these data by Sullivan et al. (1979). Due to the lack of a definitive EC₅₀ for either growth or chlorophyll content, the data are not deemed adequate for determination of a Plant Final Value.

Studies on bioconcentration of HMX were not found. Therefore no Final Residue Value can be calculated.

Because the Criterion Maximum Concentration is obtained from the Final Acute Value, it cannot be calculated. Because it is calculated as one-half of the Final Acute Value, it is unlikely to be less than 1.65 mg/L (one-half of 3.3 mg/L). Similarly, because the Criterion Continuous Concentration is equal to the least of the Chronic Value, Final Plant Value, or Final Residue Value, it cannot be determined. In summary, the minimum data base specified in USEPA guidelines (Stephan et al. 1985) for derivation of

water quality criteria for the protection of aquatic life and its uses is not now available.

HUMAN HEALTH CRITERIA

Data concerning tests for carcinogenicity of HMX were not found, and no water quality criterion based on a nonthreshold effect should be undertaken at this time. In addition, there are no human data suitable for estimating a maximum daily oral intake that produces no detectable adverse effects. Therefore the human health criterion will be calculated based on the subchronic study of Everett et al. (1985) in rats.

Acute oral toxicity to rodents was far lower than intravenous toxicity, possibly because of poor absorption from the gut. Oral LD₅₀ values ranged from 1670 mg/kg in male mice to 6440 mg/kg in female rats (Cuthbert 1985). In contrast, intravenous LD₅₀ values in rats were calculated to be 25.1 mg/kg in males and 38.1 mg/kg in females. Very similar figures were obtained by McNamara et al. (1974) in both mice and guinea pigs. The LD₅₀ by the oral route appeared to be smaller in rabbits than in rodents (Cuthbert 1985), with mortality being 2/2 in groups receiving 250 mg/kg or above.

In the 13-week mouse oral subchronic toxicity study of Everett and Maddock (1985), mortality was 25/40 for the combined groups in the 200-250 mg/kg range. At the 30 mg/kg dose level, mortality could not be statistically distinguished from controls; but some statistically significant effects were recorded: several minor changes in hematological and clinical chemistry parameters, including an elevation in albumin concentration in females, and a decrease in relative testis weight. At the 12 mg/kg dose level (males only), minor departures from control values were noted in relative testis weight and absolute kidney weight. These were significant at the $P < 0.05$ level only and were not part of any trend visible in the higher dose groups. They are best regarded as the result of data structure or the result of investigation of a large number of parameters. Thus, despite the high mortality in this study, little evidence of toxic changes could be found, and none was sufficient to give any indication of the cause of death in these animals.

In the 13-week rat study of Everett et al., (1985), with no dose-related mortality, body weight gain was reduced in a dose-related fashion. At necropsy two classes of dose-related lesions were seen (Table 8): histopathologic liver changes in males and tubular kidney change, characterized by focal atrophy and dilatation, in females. In addition there were several significant organ weight changes at higher doses, including an increase in kidney weight in females. Hematology, clinical chemistry, and urinalysis studies were performed on control and high-dose rats. Among the findings were reduced hemoglobin, slightly increased methemoglobin, increased alkaline phosphatase, and, in females, increased blood urea nitrogen and serum albumin. Some of these effects may be related to the liver and kidney damage noted above.

Based on the occurrence of toxic liver and kidney changes in the rat

study of Everett et al. (1985), (Table 8), a NOAEL of 50 mg/kg/day is suggested for males and 115 mg/kg/day for females. This is in agreement with that determined for HMX by the USEPA (McLellan et al. 1988). The smaller value, 50 mg/kg/day, is taken for calculation of the water quality criterion. This compares with a value of 0.3 mg/kg/day determined as a NOAEL for RDX by Etnier (1986), based on a 24-month dietary study.

The proposed USEPA method for the estimation of a water quality criterion for the protection of human health (USEPA 1980) is summarized in Appendix 2. When the NOAEL of 50 mg HMX/kg, taken from the chronic rat study described above (Everett et al., 1985) is used, with an uncertainty factor of 1000, the acceptable daily intake (ADI) for a 70-kg human is calculated as follows:

$$ADI = \frac{70 \text{ kg} \times \text{NOEL (mg/kg/day)}}{\text{Uncertainty factor}} = \frac{70 \times 50}{1000} = 3.5 \text{ mg/day}$$

The uncertainty factor of 1000 was chosen because the results used are from a valid long-term, but less than lifetime, feeding study on an experimental animal, in which a well defined NOAEL and LOAEL exist.

The equation for calculating a drinking water criterion for the protection of human health, given an ADI, is:

$$C = \frac{ADI - (DT + IN)}{2 \text{ L/day} + (0.0065 \text{ kg/day} \times BCF \times 1 \text{ L/kg})}$$

where

C = water quality criterion;
 ADI = acceptable daily intake, 3.5 mg/day;
 DT = dietary nonfish intake, assumed to be zero;
 IN = inhalation intake, assumed to be zero;
 L/day = daily water intake in liters;
 0.0065 = daily dietary fish intake in kg;
 BCF = bioconcentration factor, 0.49 (est. by Burrows et al. 1989; value for RDX = 4.7, Etnier 1986); and
 L/kg = unit conversion factor.

An ambient water quality criterion for the protection of human health is calculated as 1.7 mg/L. For RDX the value proposed by Etnier (1986) was 0.103 mg/L.

The studies cited above, in which toxicity to aquatic organisms was determined, showed that nominal concentrations up to 32 mg/L (actual concentrations in solution up to 3.9 mg/L) produced little or no discernible adverse effect on fish, invertebrates or algae. The proposed criterion concentration for protection of human health, 1.7 mg/L, should be protective of most aquatic organisms and their uses.

RESEARCH RECOMMENDATIONS

The following are recommended to fill gaps in the data needed to meet the USEPA requirements for generating human drinking water and aquatic life water quality criteria.

1. Chronic toxicity (lifetime) studies in rodents suitable for deriving NOAEL or LOAEL values and information on the carcinogenic potential of HMX.

2. Developmental and reproductive toxicity studies in animals.

3. An acute toxicity test on an aquatic organism outside the phyla Arthropoda and Chordata, e.g., the aquatic oligochaete annelid Lumbriculus variegatus. An acute toxicity test with an organism representing a fourth phylum, e.g., Mollusca, or an order of insect other than Diptera (represented by the midge).

4. Both acute and chronic flow-through tests using measured concentrations on three species of aquatic animals representing three families, to include (a) a fish, (b) an invertebrate, and (c) a sensitive freshwater species.

5. An acceptable test of the potential for HMX to bioconcentrate in aquatic systems.

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APPENDIX A:
SUMMARY OF USEPA METHODOLOGY FOR DERIVING NUMERICAL WATER QUALITY
CRITERIA FOR THE PROTECTION OF AQUATIC ORGANISMS AND THEIR USES

The following summary is a condensed version of the 1985 final US Environmental Protection Agency (USEPA) guidelines for calculating a water quality criteria to protect aquatic life and is slanted towards the specific regulatory needs of the US Army (e.g., discussion of saltwater aspects of the criteria calculation are not included). The guidelines are the most recent document outlining the required procedures and were written by the following researchers from the USEPA's regional research laboratories: C.E. Stephan, D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. For greater detail on individual points consult Stephan et al. (1985).

1. INTRODUCTION

The Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses describe an objective, internally consistent, and appropriate way of estimating national criteria. Because aquatic life can tolerate some stress and occasional adverse effects, protection of all species all of the time was not deemed necessary. If acceptable data are available for a large number of appropriate taxa from a variety of taxonomic and functional groups, a reasonable level of protection should be provided if all except a small fraction are protected, unless a commercially, recreationally, or socially important species was very sensitive. The small fraction is set at 0.05 because other fractions resulted in criteria that seemed too high or too low in comparison with the sets of data from which they were calculated. Use of 0.05 to calculate a Final Acute Value does not imply that this percentage of adversely affected taxa should be used to decide in a field situation whether a criterion is appropriate.

To be acceptable to the public and useful in field situations, protection of aquatic organisms and their uses should be defined as prevention of unacceptable long-term and short-term effects on (1) commercially, recreationally, and socially important species and (2) (a) fish and benthic invertebrate assemblages in rivers and streams and (b) fish, benthic invertebrate, and zooplankton assemblages in lakes, reservoirs, estuaries, and oceans. These national guidelines have been developed on the theory that effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations.

Numerical aquatic life criteria derived using these national guidelines are expressed as two numbers, so that the criteria can more accurately reflect toxicological and practical realities. The combination of a maximum concentration and a continuous concentration is designed to provide adequate protection of aquatic life and its uses from acute and chronic toxicity to animals, toxicity to plants, and

bioaccumulation by aquatic organisms without being as restrictive as a one-number criterion would have to be in order to provide the same degree of protection.

Criteria produced by these guidelines should be useful for developing water quality standards, mixing zone standards, and effluent standards. Development of such standards may have to consider additional factors such as social, legal, economic, and additional biological data. It may be desirable to derive site-specific criteria from these national criteria to reflect local conditions (USEPA 1982). The two factors that may cause the most difference between the national and site-specific criteria are the species that will be exposed and the characteristics of the water.

Criteria should provide reasonable and adequate protection with only a small possibility of considerable overprotection or underprotection. It is not enough that a criterion be the best estimate obtainable using available data; it is equally important that a criterion be derived only if adequate appropriate data are available to provide reasonable confidence that it is a good estimate. Thus, these guidelines require that certain data be available if a criterion is to be derived. If all the required data are not available, usually a criterion should not be derived; however, availability of all required data does not ensure that a criterion can be derived. The amount of guidance in these national guidelines is significant, but much of it is necessarily qualitative rather than quantitative; much judgement will be required to derive a water quality criterion for aquatic life. All necessary decisions should be based on a thorough knowledge of aquatic toxicology and an understanding of these guidelines and should be consistent with the spirit of these guidelines - which is to make best use of all available data to derive the most appropriate criterion.

2. DEFINITION OF MATERIAL OF CONCERN

1. Each separate chemical that does not ionize significantly in most natural bodies of water should be considered a separate material, except possibly for structurally similar organic compounds that only exist in large quantities as commercial mixtures of the various compounds and apparently have similar biological, chemical, physical, and toxicological properties.
2. For chemicals that do ionize significantly, all forms that would be in chemical equilibrium should usually be considered one material. Each different oxidation state of a metal and each different nonionizable covalently bonded organometallic compound should usually be considered a separate material.
3. Definition of the material should include an operational analytical component. It is also necessary to reference or

describe analytical methods that the term is intended to denote. Primary requirements of the operational analytical component is that it be appropriate for use on samples of receiving water, that it be compatible with toxicity and bioaccumulation data without making extrapolations that are too hypothetical, and that it rarely result in underprotection of aquatic life and its uses.

NOTE: Analytical chemistry of the material may have to be considered when defining the material or when judging acceptability of some toxicity tests, but a criterion should not be based on sensitivity of an analytical method. When aquatic organisms are more sensitive than analytical techniques, the proper solution is to develop better analytical methods, not to underprotect aquatic life.

3. COLLECTION OF DATA

1. Collect all available data on the material concerning (a) toxicity to, and bioaccumulation by, aquatic animals and plants; (b) FDA action levels (FDA Guidelines Manual); and (c) chronic feeding studies and long-term field studies with wildlife that regularly consume aquatic organisms.
2. All data used should be available in typed, dated and signed hardcopy with enough supporting information to indicate that acceptable test procedures were used and the results should be reliable.
3. Questionable data, whether published or not, should not be used.
4. Data on technical grade materials may be used if appropriate, but data on formulated mixtures and emulsifiable concentrates of the test material should not be used.
5. For some highly volatile, hydrolyzable, or degradable materials it may be appropriate to only use results of flow-through tests in which concentration of test material in test solutions were measured using acceptable analytical methods.
6. Do not use data obtained using brine shrimp, species that do not have reproducing wild populations in North America, or organisms that were previously exposed to significant concentrations of the test material or other contaminants.

4. REQUIRED DATA

1. Results of acceptable acute tests (see Section 5) with freshwater animals in at least eight different families such that all of the following are included:
 - a. the family Salmonidae in the class Osteichthyes;
 - b. a second family (preferably an important warmwater species) in the class Osteichthyes (e.g., bluegill, fathead minnow, or channel catfish);
 - c. a third family in the phylum Chordata (e.g. fish or amphibian);
 - d. a planktonic crustacean (e.g. cladoceran or copepod);
 - e. a benthic crustacean (e.g. ostracod, isopod, or amphipod);
 - f. an insect (e.g., mayfly, midge, stonefly);
 - g. a family in a phylum other than Arthropoda or Chordata (e.g. Annelida or Mollusca); and
 - h. a family in any order of insect or any phylum not represented.
2. Acute-chronic ratios (see Section 7) for species of aquatic animals in at least three different families provided that of the three species at least (a) one is a fish, (b) one is an invertebrate, and (c) one is a sensitive freshwater species.
3. Results of at least one acceptable test with a freshwater alga or a chronic test with a freshwater vascular plant (see Section 9). If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should be available.
4. At least one acceptable bioconcentration factor determined with an appropriate aquatic species, if a maximum permissible tissue concentration is available (see Section 10).

If all required data are available, a numerical criterion can usually be derived, except in special cases. For example, if a criterion is to be related to a water quality characteristic (see Sections 6 and 8), more data will be necessary. Similarly if all required data are not available a numerical criterion should not be derived except in special cases. For example, even if not enough acute and chronic data are available, it

may be possible to derive a criterion if the data clearly indicate that the Final Residue Value would be much lower than either the Final Chronic Value or the Final Plant Value. Confidence in a criterion usually increases as the amount of data increases. Thus, additional data are usually desirable.

5. FINAL ACUTE VALUE

1. The Final Acute Value (FAV) is an estimate of the concentration of material corresponding to a cumulative probability of 0.05 in the acute toxicity values for the genera with which acute tests have been conducted on the material. However, in some cases, if the Species Mean Acute Value (SMAV) of an important species is lower than the calculated FAV, then that SMAV replaces the FAV to protect that important species.
2. Acute toxicity tests should have been conducted using acceptable procedures (e.g., ASTM Standard E 724 or 729).
3. Generally, results of acute tests in which food was added to the test solution should not be used, unless data indicate that food did not affect test results.
4. Results of acute tests conducted in unusual dilution water, e.g., dilution water containing high levels of total organic carbon or particulate matter (higher than 5 mg/L) should not be used, unless a relationship is developed between toxicity and organic carbon or unless data show that organic carbon or particulate matter, etc. do not affect toxicity.
5. Acute values should be based on endpoints which reflect the total adverse impact of the test material on the organisms used in the tests. Therefore, only the following kinds of data on acute toxicity to freshwater aquatic animals should be used:
 - a. Tests with daphnids and other cladocerans should be started with organisms <24 hr old and tests with midges should be started with second- or third-instar larvae. The result should be the 48-hr EC50 based on percentage of organisms immobilized plus percentage of organisms killed. If such an EC50 is not available from a test, the 48-hr LC50 should be used in place of the desired 48-hr EC50. An EC50 or LC50 of longer than 48 hr can be used provided animals were not fed and control animals were acceptable at the end of the test.

- b. The result of tests with all other aquatic animal species should be the 96-hr EC50 value based on percentage of organisms exhibiting loss of equilibrium plus percentage of organisms immobilized plus percentage of organisms killed. If such an EC50 value is not available from a test, the 96-hr LC50 should be used in place of the desired EC50.
 - c. Tests with single-cell organisms are not considered acute tests, even if the duration was $\leq$ 96 hr.
 - d. If the tests were conducted properly, acute values reported as greater than values and those acute values which are above solubility of the test material are acceptable.
6. If the acute toxicity of the material to aquatic animals has been shown to be related to a water quality characteristic (e.g., total organic carbon) for freshwater species, a Final Acute Equation should be derived based on that characteristic.
 7. If the data indicate a that one or more life stages are at least a factor of 2 times more resistant than one or more other life stages of the same species, the data for the more resistant life stages should not be used in the calculation of the SMAV because a species can only be considered protected from acute toxicity if all life stages are protected.
 8. Consider the agreement of the data within and between species. Questionable results in comparison to other acute and chronic data for the species and other species in the same genus probably should not be used.
 9. For each species for which at least one acute value is available, the SMAV should be calculated as the geometric mean of all flow-through test results in which the concentration of test material were measured. For a species for which no such result is available, calculate the geometric mean of all available acute values, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial total concentrations of test material.
- NOTE:** Data reported by original investigators should not be rounded off and at least four significant digits should be retained in intermediate calculations.
10. For each genus for which one or more SMAV is available, calculate the Genus Mean Acute Value (GMAV) as the geometric mean of the SMAVs.

11. Order the GMAVs from high to low and assign ranks (R) to the GMAVs from "1" for the lowest to "N" for the highest. If two or more GMAVs are identical, arbitrarily assign them successive ranks.
12. Calculate the cumulative probability (P) for each GMAV as $R/(N+1)$.
13. Select the four GMAVs which have cumulative probabilities closest to 0.05 (if there are <59 GMAVs, these will always be the four lowest GMAVs).
14. Using the selected GMAVs and Ps, calculate

$$S^2 = \frac{\Sigma((\ln \text{GMAV})^2) - ((\Sigma(\ln \text{GMAV}))^2/4)}{\Sigma(P) - ((\Sigma(\sqrt{P}))^2/4)}$$

$$L = (\Sigma(\ln \text{GMAV}) - S(\Sigma(\sqrt{P}))) / 4$$

$$A = S(\sqrt{0.05}) + L$$

$$\text{FAV} = e^A$$

15. If for an important species, such as a recreationally or commercially important species, the geometric mean of acute values from flow-through tests in which concentrations of test material were measured is lower than the FAV, then that geometric mean should be used as the FAV.
16. Go to Section 7.

6. FINAL ACUTE EQUATION

1. When enough data show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.
2. For each species for which comparable acute toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of acute toxicity values on values of the water quality characteristic.

3. Decide whether the data for each species is useful, considering the range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used.
4. Individually for each species calculate the geometric mean of the acute values and then divide each of the acute values for a species by the mean for the species. This normalizes the acute values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0
5. Similarly normalize the values of the water quality characteristic for each species individually.
6. Individually for each species perform a least squares regression of the normalized acute toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 2. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.
7. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope (V) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.
8. For each species calculate the geometric mean (W) of the acute toxicity values and the geometric mean (X) of the related values of the water quality characteristic (calculated in 4. and 5. above).
9. For each species calculate the logarithmic intercept (Y) of the SMAV at a selected value (Z) of the water quality characteristic using the equation: $Y = \ln W - V(\ln X - \ln Z)$.
10. For each species calculate the SMAV using: $SMAV = e^Y$.

11. Obtain the FAV at Z by using the procedure described in Section 5. (No. 10-14).
12. If the SMAV for an important species is lower than the FAV at Z, then that SMAV should be used as the FAV at Z.
13. The Final Acute Equation is written as: $FAV = e^{(V[\ln(\text{water quality characteristic}) + \ln A - V[\ln Z]])}$, where V = pooled acute slope and A = FAV at Z. Because V, A, and Z are known, the FAV can be calculated for any selected value of the water quality characteristic.

7. FINAL CHRONIC VALUE

1. Depending on available data, the Final Chronic Value (FCV) might be calculated in the same manner as the FAV or by dividing the FAV by the Final Acute-Chronic Ratio.

NOTE: Acute-chronic ratios and application factors are ways of relating acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond known or estimated sensitivities of aquatic organisms. Another advantage of the acute-chronic ratio is that it should usually be greater than one; this should avoid confusion as to whether a large application factor is one that is close to unity or one that has a denominator that is much greater than the numerator.

2. Chronic values should be based on results of flow-through (except renewal is acceptable for daphnids) chronic tests in which concentrations of test material were properly measured at appropriate times during testing.
3. Results of chronic tests in which survival, growth, or reproduction in controls was unacceptably low should not be used. Limits of acceptability will depend on the species.
4. Results of chronic tests conducted in unusual dilution water should not be used, unless a relationship is developed between toxicity and the unusual characteristic or unless data show the characteristic does not affect toxicity.
5. Chronic values should be based on endpoints and exposure durations appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests should be used:

- a. Life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Tests with fish should begin with embryos or newly hatched young <48 hr old, continue through maturation and reproduction, and should end not <24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin with young <24 hr old and last for not <21 days. For fish, data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability. For daphnids, data should be obtained and analyzed on survival and young per female.
- b. Partial life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Partial life-cycle tests are allowed with fish species that require more than a year to reach sexual maturity, so that all major life stages can be exposed to the test material in less than 15 months. Exposure to the test material should begin with juveniles at least 2 months prior to active gonadal development, continue through maturation and reproduction, and should end not <24 days (90 days for salmonids) after the hatching of the next generation. Data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability.
- c. Early life-stage toxicity tests consisting of 28- to 32-day (60 days posthatch for salmonids) exposures of early life stages of a species of fish from shortly after fertilization through embryonic, larval, and early juvenile development. Data should be obtained on growth and survival.

NOTE: Results of an early life-stage test are used as predictors of results of life-cycle and partial life-cycle tests with the same species. Therefore, when results of a life-cycle or partial life-cycle test are available, results of an early life-stage test with the same species should not be used. Also, results of early life-stage tests in which the incidence of mortalities or abnormalities increased substantially near the end of the test should not be used because results of such tests may be poor estimates of results of a comparable life-cycle or partial life-cycle test.

6. A chronic value may be obtained by calculating the geometric mean of lower and upper chronic limits from a chronic test or by analyzing chronic data using regression analysis. A lower chronic limit is the highest tested concentration (a) in an acceptable chronic test, (b) which did not cause an unacceptable amount of an adverse effect on any specified biological measurements, and (c) below which no tested concentration caused such an unacceptable effect. An upper chronic limit is the lowest tested concentration (a) in an acceptable chronic test, (b) which did cause an unacceptable amount of an adverse effect on one or more of specified biological measurements, and (c) above which all tested concentrations caused such an effect.
7. If chronic toxicity of material to aquatic animals appears to be related to a water quality characteristic, a Final Chronic Equation should be derived based on that water quality characteristic. Go to Section 8.
8. If chronic values are available for species in eight families as described in Section 4 (No. 1), a Species Mean Chronic Value (SMCV) should be calculated for each species for which at least one chronic value is available by calculating the geometric mean of all chronic values for the species and appropriate Genus Mean Chronic Values should be calculated. The FCV should then be obtained using procedures described in Section 5 (No. 10-14). Then go to Section 7 (No. 13).
9. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an acute-chronic ratio, using for the numerator the geometric mean of results of all acceptable flow-through (except static is acceptable for daphnids) acute tests in the same dilution water and in which concentrations were measured. For fish, the acute test(s) should have been conducted with juveniles. Acute test(s) should have been part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted in the same laboratory and dilution water may be used. If acute tests were not conducted as part of the same study, acute tests conducted in the same dilution water but a different laboratory may be used. If such acute tests are not available, an acute-chronic ratio should not be calculated.
10. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios for that species.

11. For some materials the acute-chronic ratio is about the same for all species, but for other materials the ratio increases or decreases as the SMAV increases. Thus, the Final Acute-Chronic Ratio can be obtained in three ways, depending on the data.

- a. If the species mean acute-chronic ratio increases or decreases as the SMAV increases, the final Acute-Chronic Ratio should be calculated as the geometric mean of all species whose SMAVs are close to the FAV.
- b. If no major trend is apparent and the acute-chronic ratios for a number of species are within a factor of ten, the Final Acute-Chronic Ratio should be calculated as the geometric mean of all species mean acute-chronic ratios for both freshwater and saltwater species.
- c. If the most appropriate species mean acute-chronic ratios are <2.0 , and especially if they are <1.0 , acclimation has probably occurred during the chronic test. Because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the Final Acute-Chronic Ratio should be set at 2.0 so that the FCV is equal to the Criterion Maximum Concentration.

If the acute-chronic ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and a FCV probably cannot be calculated.

- 12. Calculate the FCV by dividing the FAV by the Final Acute-Chronic Ratio.
- 13. If the SMAV of an important species is lower than the calculated FCV, then that SMCV should be used as the FCV.
- 14. Go to Section 9.

8. FINAL CHRONIC EQUATION

- 1. A Final Chronic Equation can be derived in two ways. The procedure described in this section will result in the chronic slope being the same as the acute slope.
 - a. If acute-chronic ratios for enough species at enough values of the water quality characteristics indicate that the acute-chronic ratio is probably the same for all species and independent of the water quality

characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the species mean acute-chronic ratios.

- b. Calculate the FCV at the selected value Z of the water quality characteristic by dividing the FAV at Z by the Final Acute-Chronic Ratio.
 - c. Use V = pooled acute slope as L = pooled chronic slope.
 - d. Go to Section 8, No. 2, item m.
2. The procedure described in this section will usually result in the chronic slope being different from the acute slope.
- a. When enough data are available to show that chronic toxicity to at least one species is related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.
 - b. For each species for which comparable chronic toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of chronic toxicity values on values of the water quality characteristic.
 - c. Decide whether data for each species is useful, taking into account range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used. If a useful chronic slope is not available for at least one species or if the slopes are too dissimilar or if data are inadequate to define the relationship between chronic toxicity and water quality characteristic, return to Section 7 (No. 8), using results of tests conducted under conditions and in water similar to those commonly used for toxicity tests with the species.
 - d. For each species calculate the geometric mean of the available chronic values and then divide each chronic value for a species by the mean for the species.

This normalizes the chronic values so that the geometric mean of the normalized values for each species and for any combination of species is 1.0.

- e. Similarly normalize the values of the water quality characteristic for each species individually.
- f. Individually for each species perform a least squares regression of the normalized chronic toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 1. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.
- g. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope (L) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.
- h. For each species calculate the geometric mean (M) of toxicity values and the geometric mean (P) of related values of the water quality characteristic.
- i. For each species calculate the logarithm (Q) of the SMCVs at a selected value (Z) of the water quality characteristic using the equation: $Q = \ln M - L(\ln P - \ln Z)$.
- j. For each species calculate a SMCV at Z as the antilog of Q ($SMCV = e^Q$).
- k. Obtain the FCV at Z by using the procedure described in Section 5 (No. 10-14).
- l. If the SMCV at Z of an important species is lower than the calculated FCV at Z, then that SMCV should be used as the FCV at Z.
- m. The Final Chronic Equation is written as: $FCV = e^{(L[\ln(\text{water quality characteristic})] + \ln S - L[\ln Z])}$, where L = mean chronic slope and S = FCV at Z.

9. FINAL PLANT VALUE

1. Appropriate measures of toxicity of the material to aquatic plants are used to compare relative sensitivities of aquatic plants and animals. Although procedures for conducting and interpreting results of toxicity tests with plants are not well developed, results of such tests usually indicate that criteria which adequately protect aquatic animals and their uses also protect aquatic plants and their uses.
2. A plant value is the result of any test conducted with an alga or an aquatic vascular plant.
3. Obtain the Final Plant Value by selecting the lowest result obtained in a test on an important aquatic plant species in which concentrations of test material were measured and the endpoint is biologically important.

10. FINAL RESIDUE VALUE

1. The Final Residue Value (FRV) is intended to (a) prevent concentrations in commercially or recreationally important aquatic species from exceeding applicable FDA action levels and (b) protect wildlife, including fish and birds, that consume aquatic organisms from demonstrated unacceptable effects. The FRV is the lowest of residue values that are obtained by dividing maximum permissible tissue concentrations by appropriate bioconcentration or bioaccumulation factors. A maximum permissible tissue concentration is either (a) a FDA action level (FDA administrative guidelines) for fish oil or for the edible portion of fish or shellfish or (b) a maximum acceptable dietary intake (ADI) based on observations on survival, growth, or reproduction in a chronic wildlife feeding study or a long-term wildlife field study. If no maximum permissible tissue concentration is available, go to Section 11., because a Final Residue Value cannot be derived.
2. Bioconcentration Factors (BCFs) and Bioaccumulation Factors (BAFs) are the quotients of the concentration of a material in one or more tissues of an aquatic organism divided by the average concentration in the solution to which the organism has been exposed. A BCF is intended to account only for net uptake directly from water, and thus almost has to be measured in a laboratory test. A BAF is intended to account for net uptake from both food and water in a real-world situation, and almost has to be measured in a field situation in which predators accumulate the material directly from

water and by consuming prey. Because so few acceptable BAFs are available, only BCFs will be discussed further, but an acceptable BAF can be used in place of a BCF.

3. If a maximum permissible tissue concentration is available for a substance (e.g., parent material or parent material plus metabolite), the tissue concentration used in BCF calculations should be for the same substance. Otherwise the tissue concentration used in the BCF calculation should be that of the material and its metabolites which are structurally similar and are not much more soluble in water than the parent material.
 - a. A BCF should be used only if the test was flow-through, the BCF was calculated based on measured concentrations of test material in tissue and in the test solution, and exposure continued at least until either apparent steady-state (BCF does not change significantly over a period of time, such as two days or 16 percent of exposure duration, whichever is longer) or 28 days was reached. The BCF used from a test should be the highest of (a) the apparent steady-state BCF, if apparent steady-state was reached; (b) highest BCF obtained, if apparent steady-state was not reached; and (c) projected steady-state BCF, if calculated.
 - b. Whenever a BCF is determined for a lipophilic material, percentage of lipids should also be determined in the tissue(s) for which the BCF is calculated.
 - c. A BCF obtained from an exposure that adversely effected the test organisms may be used only if it is similar to that obtained with unaffected individuals at lower concentrations that did cause effects.
 - d. Because maximum permissible tissue concentrations are rarely based on dry weights, a BCF calculated using dry tissue weights must be converted to a wet tissue weight basis. If no conversion factor is reported with the BCF, multiply the dry weight by 0.1 for plankton and by 0.2 for species of fishes and invertebrates.
 - e. If more than one acceptable BCF is available for a species, the geometric mean of values should be used, unless the BCFs are from different exposure durations, then the BCF for the longest exposure should be used.

4. If enough pertinent data exist, several residue values can be calculated by dividing maximum permissible tissue concentrations by appropriate BCFs:
 - a. For each available maximum ADI derived from a feeding study or a long-term field study with wildlife, including birds and aquatic organisms, the appropriate BCF is based on the whole body of aquatic species which constitute or represent a major portion of the diet of tested wildlife species.
 - b. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.
5. For lipophilic materials, it may be possible to calculate additional residue values. Because the steady-state BCF for a lipophilic material seems to be proportional to percentage of lipids from one tissue to another and from one species to another (Hamelink et al. 1971, Lundsford and Blem 1982, Schnoor 1982), extrapolations can be made from tested tissues or species to untested tissues or species on the basis of percentage of lipids.
 - a. For each BCF for which percentage of lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a one percent lipid basis by dividing the BCF by percentage of lipids. This adjustment makes all the measured BCFs comparable regardless of species or tissue.
 - b. Calculate the geometric mean normalized BCF.
 - c. Calculate all possible residue values by dividing available maximum permissible tissue concentrations by the mean normalized BCF and by the percentage of lipids values appropriate to the maximum permissible tissue concentration.
 - For an FDA action level for fish oil, the appropriate percentage of lipids value is 100.
 - For an FDA action level for fish, the appropriate percentage of lipids value is 11 for freshwater criteria, based on the highest levels for important consumed species (Sidwell 1981).

- For a maximum ADI derived from a chronic feeding study or long-term field study with wildlife, the appropriate percentage of lipids is that of an aquatic species or group of aquatic species which constitute a major portion of the diet of the wildlife species.

6. The FRV is obtained by selecting the lowest of available residue values.

11. OTHER DATA

Pertinent information that could not be used in earlier sections may be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other biologically important adverse effect. Especially important are data for species for which no other data are available.

12. CRITERION

1. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.
2. The Criterion Maximum Concentration (CMC) is equal to one-half of the FAV.
3. The Criterion Continuous Concentration (CCC) is equal to the lower of the FCV, the Final Plant Value, and the FRV unless other data show a lower value should be used. If toxicity is related to a water quality characteristic, the CCC is obtained from the Final Chronic Equation, the Final Plant Value, and the FRV by selecting the value or concentration that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see Section 11) show that a lower value should be used.
4. Round both the CCC and CMC to two significant figures.

5. The criterion is stated as:

The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that (except possibly where a locally important species is very sensitive) (1) aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of (2) does not exceed (3) $\mu\text{g/L}$ more than once every three years on the average and if the one-hour average concentration does not exceed (4) $\mu\text{g/L}$ more than once every three years on the average.

Where

- (1) = insert freshwater or saltwater,
- (2) = name of material,
- (3) = insert the Criterion Continuous Concentration, and
- (4) = insert the Criterion Maximum Concentration.

13. REFERENCES

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APPENDIX B:
SUMMARY OF USEPA METHODOLOGY FOR DETERMINING WATER QUALITY
CRITERIA FOR THE PROTECTION OF HUMAN HEALTH

The following summary is a condensed version of the 1980 final US Environmental Protection Agency (USEPA) guidelines for calculating a water quality criteria to protect human health and is slanted towards the specific regulatory needs of the US Army. The guidelines are the most recent document outlining the required procedures and were published in the Federal Register (USEPA 1980). For greater detail on individual points consult that reference.

1. INTRODUCTION

The EPA's water quality criteria for the protection of human health are based on one or more of the following properties of a chemical pollutant:

- a) Carcinogenicity, b) Toxicity, and c) Organoleptic (taste and odor) effects.

The meanings and practical uses of the criteria values are distinctly different depending on the properties on which they are based. Criteria based solely on organoleptic effects do not necessarily represent approximations of acceptable risk levels for human health. In all other cases the criteria values represent either estimations of the maximum allowable ambient water concentrations of a pollutant which would prevent adverse health effects or, for suspect and proven carcinogens, estimations of the increased cancer risk associated with incremental changes in the ambient water concentration of the substance. Social and economic costs and benefits are not considered in determining water quality criteria. In establishing water quality standards, the choice of the criterion to be used depends on the designated water use. In the case of a multiple-use water body, the criterion protecting the most sensitive use is applied.

2. DATA NEEDED FOR HUMAN HEALTH CRITERIA

Criteria documentation requires information on: (1) exposure levels, (2) pharmacokinetics, and (3) range of toxic effects of a given water pollutant.

2.1 EXPOSURE DATA

For an accurate assessment of total exposure to a chemical, consideration must be given to all possible exposure routes including ingestion of contaminated water and edible aquatic and nonaquatic organisms, as well as exposure through inhalation and dermal contact. For water quality criteria the most important exposure routes to be considered are ingestion of water and consumption of fish and shellfish.

Generally, exposure through inhalation, dermal contact, and non-aquatic diet is either unknown or so low as to be insignificant; however, when such data are available, they must be included in the criteria evaluation.

The EPA guidelines for developing water quality criteria are based on the following assumptions which are designed to be protective of a healthy adult male who is subject to average exposure conditions:

1. The exposed individual is a 70-kg male person (International Commission on Radiological Protection 1977).
2. The average daily consumption of freshwater and estuarine fish and shellfish products is equal to 6.5 grams.
3. The average daily ingestion of water is equal to 2 liters (Drinking Water and Health, National Research Council 1977).

Because fish and shellfish consumption is an important exposure factor, information on bioconcentration of the pollutant in edible portions of ingested species is necessary to calculate the overall exposure level. The bioconcentration factor (BCF) is equal to the quotient of the concentration of a substance in all or part of an organism divided by the concentration in ambient water to which the organism has been exposed. The BCF is a function of lipid solubility of the substance and relative amount of lipids in edible portions of fish or shellfish. To determine the weighted average BCF, three different procedures can be used depending upon lipid solubility and availability of bioconcentration data:

(1) For lipid soluble compounds, the average BCF is calculated from the weighted average percent lipids in ingested fish and shellfish in the average American diet. The latter factor has been estimated to be 3 percent (Stephan 1980, as cited in USEPA 1980)

Because steady-state BCFs for lipid soluble compounds are proportional to percent lipids, the BCF for the average American diet can be calculated as follows:

$$BCF_{avg} = BCF_{sp} \times \frac{3.0\%}{PL_{sp}}$$

where BCF_{sp} is the bioconcentration factor for an aquatic species and PL_{sp} is the percent lipids in the edible portions of that species.

(2) Where an appropriate bioconcentration factor is not available, the BCF can be estimated from the octanol/water partition coefficient (P) of a substance as follows:

$$\log BCF = (0.85 \log P) - 0.70$$

for aquatic organisms containing about 7.6 percent lipids (Veith et al. 1980, as cited in USEPA 1980). An adjustment for percent

lipids in the average diet (3 percent versus 7.6 percent) is made to derive the weighted average bioconcentration factor.

(3) For nonlipid-soluble compounds, the available BCFs for edible portions of consumed freshwater and estuarine fish and shellfish are weighted according to consumption factors to determine the weighted BCF representative of the average diet.

2.2 PHARMACOKINETIC DATA

Pharmacokinetic data, encompassing information on absorption, distribution, metabolism, and excretion, are needed for determining the biochemical fate of a substance in human and animal systems. Information on absorption and excretion in animals, together with a knowledge of ambient concentrations in water, food, and air, are useful in estimating body burdens in humans. Pharmacokinetic data are also essential for estimating equivalent oral doses based on data from inhalation or other routes of exposure.

2.3 BIOLOGICAL EFFECTS DATA

Effects data which are evaluated for water quality criteria include acute, subchronic, and chronic toxicity; synergistic and antagonistic effects; and genotoxicity, teratogenicity, and carcinogenicity. The data are derived primarily from animal studies, but clinical case histories and epidemiological studies may also provide useful information. According to the EPA (USEPA 1980), several factors inherent in human epidemiological studies often preclude their use in generating water quality criteria (see NAS 1977). However, epidemiological data can be useful in testing the validity of animal-to-man extrapolations.

From an assessment of all the available data, a biological endpoint, i.e., carcinogenicity, toxicity, or organoleptic effects is selected for criteria formulation.

3. HUMAN HEALTH CRITERIA FOR CARCINOGENIC SUBSTANCES

If sufficient data exist to conclude that a specific substance is a potential human carcinogen (carcinogenic in animal studies, with supportive genotoxicity data, and possibly also supportive epidemiological data) then the position of the EPA is that the water quality criterion for that substance (recommended ambient water concentration for maximum protection of human health) is zero. This is because the EPA believes that no method exists for establishing a threshold level for carcinogenic effects, and, consequently, there is no scientific basis for establishing a "safe" level. To better define the carcinogenic risk associated with a particular water pollutant, the EPA has developed a methodology for determining ambient water concentrations of the substance which would correspond to incremental lifetime cancer risks of 10^{-7} to

10⁻⁵ (one additional case of cancer in populations ranging from ten million to 100,000, respectively). These risk estimates, however, do not represent an EPA judgment as to an "acceptable" risk level.

3.1 METHODOLOGY FOR DETERMINING CARCINOGENICITY (NONTHRESHOLD) CRITERIA

The ambient water concentration of a substance corresponding to a specific carcinogenic risk can be calculated as follows:

$$C = \frac{70 \times PR}{q_1^* (2 + 0.0065 BCF)}$$

where,

C = ambient water concentration;

PR = the probable risk (e.g., 10⁻⁵; equivalent to one case in 100,000);

BCF = the bioconcentration factor; and

q₁^{*} = a coefficient (defined below) (USEPA 1980).

By rearranging the terms in this equation, it can be seen that the ambient water concentration is one of several factors which define the overall exposure level:

$$PR = q_1^* \times \frac{C (2 + 0.0065 BCF)}{70}$$

or

$$PR = q_1^* \times \frac{2C + (0.0065 BCF \times C)}{70}$$

where, 2C is the daily exposure resulting from drinking 2 liters of water per day and (0.0065 x BCF x C) is the average daily exposure resulting from the consumption of 6.5 mg of fish and shellfish per day. Because the exposure is calculated for a 70-kg man, it is normalized to a per kilogram basis by the factor of 1/70. In this particular case, exposure resulting from inhalation, dermal contact, and nonaquatic diet is considered to be negligible.

In simplified terms the equation can be rewritten

$$PR = q_1^* X,$$

where X is the total average daily exposure in mg/kg/day

or

$$q_1^* = \frac{PR}{X},$$

showing that the coefficient q₁^{*} is the ratio of risk to dose; an indication of the carcinogenic potency of the compound.

The USEPA guidelines state that for the purpose of developing water quality criteria, the assumption is made that at low dose levels there

is a linear relationship between dose and risk (at high doses, however, there may be a rapid increase in risk with dose resulting in a sharply curved dose/response curve). At low doses then, the ratio of risk to dose does not change appreciably and q_1^* is a constant. At high doses the carcinogenic potency can be derived directly from experimental data, but for risk levels of 10^{-7} to 10^{-5} , which correspond to very low doses, the q_1^* value must be derived by extrapolation from epidemiological data or from high dose, short-term animal bioassays.

3.2 CARCINOGENIC POTENCY CALCULATED FROM HUMAN DATA

In human epidemiological studies, carcinogenic effect is expressed in terms of the relative risk [RR(X)] of a cohort of individuals at exposure X compared to the risk in the control group [PR(control)] (e.g., if the cancer risk in group A is five times greater than that of the control group, then $RR(X) = 5$). In such cases the "excess" relative cancer risk is expressed as $RR(X) - 1$, and the actual numeric, or proportional excess risk level [PR(X)] can be calculated:

$$PR(X) = [RR(X) - 1] \times PR(\text{control}).$$

Using the standard risk/dose equation:

$$PR(X) = b \times X$$

and substituting for PR(X):

$$[RR(X) - 1] \times PR(\text{control}) = b \times X$$

or

$$b = \frac{[RR(X) - 1] \times PR(\text{control})}{X}$$

where b is equal to the carcinogenic potency or q_1^* .

3.3 CARCINOGENIC POTENCY CALCULATED FROM ANIMAL DATA

In the case of animal studies where different species, strains, and sexes may have been tested at different doses, routes of exposure, and exposure durations, any data sets used in calculating the health criteria must conform to certain standards:

1. The tumor incidence must be statistically significantly higher than the control for at least one test dose level and/or the tumor incidence rate must show a statistically significant trend with respect to dose level.
2. The data set giving the highest estimate of carcinogenic lifetime risk (q_1^*) should be selected unless the sample size is quite small and another data set with a similar dose-response relationship and larger sample size is available.

3. If two or more data sets are comparable in size and identical with respect to species, strain, sex, and tumor site, then the geometric mean of q_1^* from all data sets is used in the risk assessment.
4. If in the same study tumors occur at a significant frequency at more than one site, the cancer incidence is based on the number of animals having tumors at any one of those sites.

In order to make different data sets comparable, the EPA guidelines call for the following standardized procedures:

1. To establish equivalent doses between species, the exposures are normalized in terms of dose per day (m) per unit of body surface area. Because the surface area is proportional to the $2/3$ power of the body weight (W), the daily exposure (X) can be expressed as:

$$X = \frac{m}{W^{2/3}}.$$

2. If the dose (s) is given as mg per kg of body weight:

$$S = \frac{m}{W},$$

then

$$m = s \times W$$

and the equivalent daily exposure (X) would be

$$X = \frac{(s \times W)}{W^{2/3}}$$

or

$$X = s \times W^{1/3}.$$

3. The dose must also be normalized to a lifetime average exposure. For an carcinogenic assay in which the average dose per day (in mg) is m , and the length of exposure is l_e , and the total length of the experiment is L_e , then the lifetime average exposure (X_m) is:

$$X_m = \frac{l_e \times m}{L_e \times W^{2/3}}.$$

4. If the duration of the experiment (L_0) is less than the natural life span (L) of the test animal, the value of q_1^* is increased by a factor of $(L/L_0)^3$ to adjust for an age-specific increase in the cancer rate.

5. If the exposure is expressed as the dietary concentration of a substance (in ppm), then the dose per day (m) is

$$m = \text{ppm} \times F \times r,$$

where F is the weight of the food eaten per day in kg, and r is the absorption fraction (which is generally assumed to be equal to 1). The weight of the food eaten per day can be expressed as a function of body weight

$$F = fW,$$

where f is a species-specific, empirically derived coefficient which adjusts for differences in F due to differences in the caloric content of each species diet (f is equal to 0.028 for a 70-kg man; 0.05 for a 0.35-kg rat; and 0.13 for a 0.03-kg mouse).

Substituting ($\text{ppm} \times F$) for m and fW for F , the daily exposure (dose/surface area/day or $m/W^{2/3}$) can be expressed as

$$X = \frac{\text{ppm} \times F}{W^{2/3}} = \frac{\text{ppm} \times f \times W}{W^{2/3}} = \text{ppm} \times f \times W^{1/3}.$$

6. When exposure is via inhalation, calculation can be considered for two cases: (1) the substance is a water soluble gas or aerosol, and is absorbed proportionally to the amount of air breathed in and (2) the substance is not very water soluble and absorption, after equilibrium is reached between the air and the body compartments, will be proportional to the metabolic rate which is proportional to rate of oxygen consumption; which, in turn, is a function of total body surface area.

3.4 EXTRAPOLATION FROM HIGH TO LOW DOSES

Once experimental data have been standardized in terms of exposure levels, they are incorporated into a mathematical model which allows for calculation of excess risk levels and carcinogenic potency at low doses by extrapolation from high dose situations. There are a number of mathematical models which can be used for this procedure (see Krewski et al. 1983 for review). The EPA has selected a "linearized multi-stage" extrapolation model for use in deriving water quality criteria (USEPA 1980). This model is derived from a standard "general product" time-to-response (tumor) model (Krewski et al. 1983):

$$P(t;d) = 1 - \exp\{-g(d)H(t)\},$$

where $P(t;d)$ is the probable response for dose d and

time t ; $g(d)$ is the polynomial function defining the effect of dose level, and $H(t)$ the effect of time:

$$g(d) = \sum_{i=0}^a \alpha_i d^i,$$

$$H(t) = \sum_{i=1}^b \beta_i t^i,$$

(with α and $\beta \geq 0$, and $\sum \beta_i = 1$).

This time-to-response model can be converted to a quantal response model by incorporation of the time factor into each α as a multiplicative constant (Crump 1980):

$$P(d/t) = 1 - \exp\left\{-\sum_{i=0}^a \alpha_i d^i\right\},$$

or as given in the EPA guidelines (USEPA 1980):

$$P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \dots + q_k d^k)],$$

where $P(d)$ is the lifetime risk (probability) of cancer at dose d .

For a given dose the excess cancer risk $A(d)$ above the background rate $P(0)$ is given by the equation:

$$A(d) = \frac{P(d) - P(0)}{1 - P(0)},$$

where

$$A(d) = 1 - \exp[-q_1 d + q_2 d^2 + \dots + q_k d^k],$$

Point estimates of the coefficients $q_1 \dots q_k$ and consequently the extra risk function $A(d)$ at any given dose are calculated by using the statistical method of maximum likelihood. Whenever q_1 is not equal to 0, at low doses the extra risk function $A(d)$ has approximately the form:

$$A(d) = q_1 \times d.$$

Consequently, $q_1 \times d$ represents a 95 percent upper confidence limit on the excess risk, and R/q_1 represents a 95 percent lower confidence limit on the dose producing an excess risk of R . Thus $A(d)$ and R will be a function of the maximum possible value of q_1 which can be determined from the 95 percent upper confidence limits on q_1 . This is accomplished by using the computer program GLOBAL 79 developed by Crump and Watson (1979). In this procedure q_1^* , the 95 percent upper confidence limit, is calculated by increasing q_1 to a value which, when incorporated into the log-likelihood function, results in a maximum value satisfying the equation:

$$2(L_0 - L_1) = 2.70554,$$

where L_0 is the maximum value of the log-likelihood function.

Whenever the multistage model does not fit the data sufficiently, data at the highest dose are deleted and the model is refitted to the data. To determine whether the fit is acceptable, the chi-square statistic is used:

$$\chi^2 = \sum_{i=1}^h \frac{(X_i - N_i P_i)^2}{N_i P_i \times (1 - P_i)},$$

where N_i is the number of animals in the i th dose group, X_i is the number of animals in the i th dose group with a tumor response, P_i is the probability of a response in the i th dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups.

The fit is determined to be unacceptable whenever chi-square (χ^2) is larger than the cumulative 99 percent point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of nonzero multistage coefficients.

4. HEALTH CRITERIA FOR NONCARCINOGENIC TOXIC SUBSTANCES

Water quality criteria that are based on noncarcinogenic human health effects can be derived from several sources of data. In all cases it is assumed that the magnitude of a toxic effect decreases as the exposure level decreases until a threshold point is reached at, and below which, the toxic effect will not occur regardless of the length of the exposure period. Water quality criteria (C) establish the concentration of a substance in ambient water which, when considered in relation to other sources of exposure [i.e., average daily consumption of nonaquatic organisms (DT) and daily inhalation (IN)], place the Acceptable Daily Intake (ADI) of the substance at a level below the toxicity threshold, thereby preventing adverse health effects:

$$C = \frac{ADI - (DT + IN)}{[2L + (0.0065 \text{ kg} \times BCF)]}$$

where 2L is the amount of water ingested per day, 0.0065 kg is the amount of fish and shellfish consumed per day, and BCF is the weighted average bioconcentration factor.

In terms of scientific validity, an accurate estimate of the ADI is the major factor in deriving a satisfactory water quality criteria.

The threshold exposure level, and thus the ADI, can be derived from either or both animal and human toxicity data.

4.1 NONCARCINOGENIC HEALTH CRITERIA BASED ON ANIMAL TOXICITY DATA (ORAL)

For criteria derivation, toxicity is defined as any adverse effects which result in functional impairment and/or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge (USEPA 1980).

A bioassay yielding information as to the highest chronic (90 days or more) exposure tolerated by the test animal without adverse effects (No-Observed-Adverse-Effect-Level or NOAEL) is equivalent to the toxicity threshold and can be used directly for criteria derivation. In addition to the NOAEL, other data points which can be obtained from toxicity testing are

- (1) NOEL = No-Observed-Effect-Level,
- (2) LOEL = Lowest-Observed-Effect-Level,
- (3) LOAEL = Lowest-Observed-Adverse-Effect-Level,
- (4) FEL = Frank-Effect-Level.

According to the EPA guidelines, only certain of these data points can be used for criteria derivation:

1. A single FEL value, without information on the other response levels, should not be used for criteria derivation because there is no way of knowing how far above the threshold it occurs.
2. A single NOEL value is also unsuitable because there is no way of determining how far below the threshold it occurs. If only multiple NOELs are available, the highest value should be used.
3. If a LOEL value alone is available, a judgement must be made as to whether the value actually corresponds to a NOAEL or an LOAEL.
4. If an LOAEL value is used for criteria derivation, it must be adjusted by a factor of 1 to 10 to make it approximately equivalent to the NOAEL and thus the toxicity threshold.
5. If for reasonably closely spaced doses only a NOEL and a LOAEL value of equal quality are available, the NOEL is used for criteria derivation.

The most reliable estimate of the toxicity threshold would be one obtained from a bioassay in which an NOEL, NOAEL, LOAEL, and clearly defined FEL were observed in relatively closely spaced doses.

Regardless of which of the above data points is used to estimate the toxicity threshold, a judgement must be made as to whether the experimental data are of satisfactory quality and quantity to allow for a valid extrapolation for human exposure situations. Depending on whether the data are considered to be adequate or inadequate, the

toxicity threshold is adjusted by a "safety factor" or "uncertainty factor" (NAS 1977). The "uncertainty factor" may range from 10 to 1000 according to the following general guidelines:

1. Uncertainty factor 10. Valid experimental results from studies on prolonged ingestion by man, with no indication of carcinogenicity.
2. Uncertainty factor 100. Data on chronic exposures in humans not available. Valid results of long-term feeding studies on experimental animals, or in the absence of human studies, valid animal studies on one or more species. No indication of carcinogenicity.
3. Uncertainty factor 1000. No long-term or acute exposure data for humans. Scanty results on experimental animals with no indication of carcinogenicity.

Uncertainty factors which fall between the categories described above should be selected on the basis of a logarithmic scale (e.g., 33 being halfway between 10 and 100).

The phrase "no indication of carcinogenicity" means that carcinogenicity data from animal experimental studies or human epidemiology are not available. Data from short-term carcinogenicity screening tests may be reported, but they are not used in criteria derivation or for ruling out the uncertainty factor approach.

4.2 CRITERIA BASED ON INHALATION EXPOSURES

In the absence of oral toxicity data, water quality criteria for a substance can be derived from threshold limit values (TLVs) established by the American Conference of Governmental and Industrial Hygienists (ACGIH), the Occupational Safety and Health Administration (OSHA), or the National Institute for Occupational Safety and Health (NIOSH), or from laboratory studies evaluating the inhalation toxicity of the substance in experimental animals. TLVs represent 8-hr time-weighted averages of concentrations in air designed to protect workers from various adverse health effects during a normal working career. To the extent that TLVs are based on sound toxicological evaluations and have been protective in the work situation, they provide helpful information for deriving water quality criteria. However, each TLV must be examined to decide if the data it is based on can be used for calculating a water quality criteria (using the uncertainty factor approach). Also the history of each TLV should be examined to assess the extent to which it has resulted in worker safety. With each TLV, the types of effects against which it is designed to protect are examined in terms of its relevance to exposure from water. It must be shown that the chemical is not a localized irritant and there is no significant effect at the portal of entry, regardless of the exposure route.

The most important factor in using inhalation data is in determining equivalent dose/response relationships for oral exposures. Estimates of equivalent doses can be based upon (1) available pharmacokinetic data for oral and inhalation routes, (2) measurements of absorption efficiency from ingested or inhaled chemicals, or (3) comparative excretion data when associated metabolic pathways are equivalent to those following oral ingestion or inhalation. The use of pharmacokinetic models is the preferred method for converting from inhalation to equivalent oral doses.

In the absence of pharmacokinetic data, TLVs and absorption efficiency measurements can be used to calculate an ADI value by means of the Stokinger and Woodward (1958) model:

$$ADI = TLV \times BR \times DE \times d \times AA / (AO \times SF),$$

where,

BR = daily air intake (assume 10 m³),
 DE = duration of exposure in hours per day,
 d = 5 days/7 days,
 AA = efficiency of absorption from air,
 AO = efficiency of absorption from oral exposure, and
 SF = safety factor.

For deriving an ADI from animal inhalation toxicity data, the equation is:

$$ADI = CA \times DE \times d \times AA \times BR \times 70 \text{ kg} / (BWA \times AO \times SF),$$

where,

CA = concentration in air (mg/m³),
 DE = duration of exposure (hr/day),
 d = number of days exposed/number of days observed,
 AA = efficiency of absorption from air,
 BR = volume of air breathed (m³/day),
 70 kg = standard human body weight,
 BWA = body weight of experimental animals (kg),
 AO = efficiency of absorption from oral exposure, and
 SF = safety factor.

The safety factors used in the above equations are intended to account for species variability. Consequently, the mg/surface area/day conversion factor is not used in this methodology.

5. ORGANOLEPTIC CRITERIA

Organoleptic criteria define concentrations of substances which impart undesirable taste and/or odor to water. Organoleptic criteria are based on aesthetic qualities alone and not on toxicological data, and therefore have no direct relationship to potential adverse human health effects. However, sufficiently intense organoleptic effects may,

under some circumstances, result in depressed fluid intake which, in turn, might aggravate a variety of functional diseases (i.e., kidney and circulatory diseases).

For comparison purposes, both organoleptic criteria and human health effects criteria can be derived for a given water pollutant; however, it should be explicitly stated in the criteria document that the organoleptic criteria have no demonstrated relationship to potential adverse human health effects.

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