

U.S. Army Center for Health Promotion
and Preventive Medicine

**Wildlife Toxicity Assessment for
High Melting Explosive (HMX)**

NOVEMBER 2001

**Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program**

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Readiness Thru Health

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Department of the Army
U.S. Army Center for Health Promotion and Preventive Medicine

Wildlife Toxicity Assessment for HMX

CAS No. 2691-41-0

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1. INTRODUCTION

High Melting Explosive (HMX) is one of several compounds that have been released to the environment during the manufacture of explosives and in load, assembly and pack (LAP) activities at U.S. Army ammunition plants (AAPs) and other military installations. Structurally, the compound (Chemical Abstract Services Registry Number 2691-41-0) is a completely N-nitrated, eight-member heterocyclic ring compound with the empirical formula, $C_4H_8N_8O_8$. In addition to "HMX," it is known by various systematic and trivial names such as cyclotetramethylenetetranitramine, octahydro-1,3,5,7-tetranitro, 1,3,5,7-tetrazocine and octogen, among others. This Wildlife Toxicity Assessment summarizes current knowledge of the likely harmful impacts of HMX on wildlife, emphasizing threshold doses for the onset of toxicological effects, as described in reports of experimental studies of HMX. Surveying the threshold dosimetry of the compound may point to the establishment of toxicity reference values (TRVs) that could serve as protective exposure standards for wildlife ranging in the vicinity of affected sites. The protocol for the performance of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, the *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000).

2. TOXICITY PROFILE

2.1 Literature Review

Relevant biomedical, toxicological and ecological databases were electronically searched May 5, 2000, using DIALOG to identify primary reports of studies and reviews on the toxicology of HMX. Separate searches were carried out linking the compound to either laboratory mammals, birds, reptiles and amphibians (combined) and wild mammals. In general, a two-tiered approach was used in which all citations were first evaluated as titles and "key words in context." All available abstracts from articles selected in the first tier as possibly relevant to TRV development were then evaluated for relevancy and retention for evaluation in the second tier. For HMX, 17 articles were marked for retrieval from 32 initial hits.

In addition to DIALOG searching, a number of U.S. Army reports were identified in the Defense Technical Information Center. Secondary references and sources of information on HMX included an Agency for Toxic Substances and Disease Registry (ATSDR) *Toxicological Profile for HMX* (ATSDR, 1997), the National Library of Medicine's Hazardous Substances Databank (HSDB, 2000), the U.S. Environmental Protection Agency's (USEPA) Integrated Risk Information System (IRIS) (USEPA, 2000) and Health Effects Assessment Summary Tables (HEAST) (USEPA, 1997). Details concerning the search terms are presented in Appendix A.

2.2 Environmental Fate and Transport

HMX, a more powerful explosive than trinitrotoluene (TNT), has been used as a trigger mechanism for atomic (fission) weapons, as a component in plastic explosives, and in rocket fuels (ATSDR, 1997; USEPA, 1988). The compound's manufacture is limited to a single location in the United States (the Holston Plant at Kingsport, Tennessee), where it has been reported that, typically, up to 45 lb/day will be released to surrounding water bodies in discharged wastewaters from manufacturing and processing. Concentrations of HMX of up to 3.36 mg/L have been detected in effluents from the Holston facility (Talmage et al., 1999). Releases of HMX have also occurred at facilities where munitions are assembled, stored or tested. For example, concentrations of the compound of up to 5700 mg/kg have been reported in soil at some army sites (Talmage et al., 1999). Physicochemical properties of HMX relevant to the environmental fate and transport of the compound are listed in Table 1.

Table 1. Summary of Physical-Chemical Properties of HMX

Molecular weight	296.16
Color	colorless
Physical state	crystalline solid
Melting point	276–280 °C
Boiling point	no data
Odor	no data
Solubility water	5–6.63 mg/L at 20–25 °C; soluble in acetone, cyclohexanone, acetic anhydride, dimethyl sulfoxide
Partition coefficients:	
Log K_{ow}	0.06, 0.26
Log K_{oc}	0.54
Vapor pressure at 25 °C	3.33×10^{-14} mm Hg
Henry's Law constant at 25 °C	2.60×10^{-15} atm.m ³ /mole
Conversion factors	1 ppm = 12.11 mg/m ³ 1 mg/m ³ = 0.083 ppm

Sources: USEPA, 1988; ATSDR, 1997; Talmage et al., 1999; HSDB, 2000

The vapor pressure and Henry's Law constant are sufficiently low (3.33×10^{-14} mm Hg and 2.60×10^{-15} atm.m³/mole, respectively) suggesting that HMX is very unlikely to enter the air as a vapor. However, aerial dispersion of the compound while adhering to soil or dust particles has been implicated as a likely mechanism by which the compound can be released to the atmosphere (ATSDR, 1997). With a low log soil organic carbon-water partition coefficient of 0.54, HMX has the potential for high mobility in soil and could leach to ground water. For example, HMX has been detected in ground water at the Louisiana AAP at concentrations up to 4.2 mg/L (Talmage et al., 1999).

Photolysis appears to be the dominant process by which HMX is broken down in the environment, with a reported first order photolytic rate constant of 0.15 days⁻¹ (USEPA, 1988). This suggests that an aqueous concentration of 0.5 mg/L HMX will have a half-life of 4–5 days when exposed to natural sunlight. Primary products of this process include nitrate, nitrite, and formaldehyde. By contrast, biodegradation/biotransformational processes involving bacteria or other microflora are extremely slow, though the formation of 1,1-dimethyl hydrazine has been demonstrated as a result of anaerobic degradation (USEPA, 1988).

2.3 Summary of Mammalian Toxicity

2.3.1 Mammalian Toxicity – Oral

2.3.1.1 Mammalian Oral Toxicity - Acute

In one of a series of studies carried out for the U.S. Army Medical Research and Development Command by Inveresk Research International (IRI), Cuthbert et al. (1985) reported data obtained from various short-term toxicological tests on HMX. These included guinea pig sensitization, eye and skin irritation studies in rabbits, dermal and intravenous lethality in rats and rabbits, and acute oral lethality studies in rats, mice, and rabbits. In the latter, acute oral LD₅₀ values of 6.5, 2.0, and between 0.1-0.25 g/kg were found for male Fischer 344 (F344) rats, B6C3F1 mice, and New Zealand white rabbits, respectively. Female oral LD₅₀ values for rats and mice were reported as 7.6 and 3.8 g/kg, respectively. The rodent studies were conducted using five animals/sex/group. However, the rabbit investigations used one animal/sex/group at 2000, 1000, 429, 250, 100, and 50 mg/kg. Females died at each dose level. Males died at the 250, 429, 1000, and 2000 mg/kg dose level. While generally indicating that the compound has a low acute toxicity via the oral route, these data suggest the potential for wide interspecies variation.

In a further summary of the IRI data, Wilson (1985) supplemented these findings with toxicokinetic information that had been obtained by administering ¹⁴C-labeled HMX by either gavage or intravenous injection to rats and mice. For either experimental animal species, the data indicate a low level of gastrointestinal absorption of unchanged HMX in rodents. For example, in the rats, a total of 85% of the

administered dose had accumulated in the feces 4 days after dosing. Furthermore, the comparative levels of radioactivity released to the urine following intravenous versus oral administration of ^{14}C -HMX suggested that less than 5% of the oral dose of the compound had crossed the gastrointestinal absorption barrier. This result is consistent with the low oral lethality reported by Cuthbert et al. (1985). According to Wilson (1985), the little tissue deposition that had occurred was found in the liver, kidney, and brain.

2.3.1.2 Mammalian Oral Toxicity – Subacute

Greenhough and McDonald (1985a,b) published two reports on behalf of the U.S. Army in which the 14-day oral toxicity of HMX was determined in F344 rats and B6C3F1 mice. These were essentially range-finding studies for subsequent investigations of the subchronic (90-day) toxicity of this compound in these species. In the first study, the authors exposed six rats/sex/group to HMX for 14 days as a dietary addition at target doses of 0, 333, 1000, 3000 and 9000 mg/kg-day (Greenhough and McDonald 1985a). As tabulated by the authors, the actual achieved average doses equivalent to these levels were 0, 335.2, 957.4, 2981, and 8504.3 mg/kg-day in males and 0, 369.2, 1280, 3474.25, and 3055 mg/kg-day in females. In the in-life phase of the study, animals were checked daily for mortality and clinical signs, twice weekly for body weight and once weekly for food and water consumption. At termination, blood samples were taken from all animals and stored frozen. All carcasses were subjected to a gross necropsy, liver and kidney weights were recorded, while excised pieces of brain, heart, kidney, liver, spleen, and thymus were processed for histopathological examination.

Concomitant with the incremental range of HMX doses, the group-specific incidence of compound-related fatalities was 0/6 (controls), 0/6, 0/6, 0/6 and 5/6 (high-dose) in males and 0/6, 0/6, 1/6, 1/6 and 6/6 in females. These deaths were accompanied by the onset of profound clinical signs characteristic of toxicologically challenged animals, most notably in males at the two highest dose levels, but in all groups of female rats. All HMX-treated male rats displayed dose-related suppression of body weight gain, while the two highest groups showed an actual body weight loss after 4 days of exposure. This food consumption-related deficit had partially rebounded by day 7. All females receiving HMX showed an initial body weight loss to levels that stayed depressed compared to initial values for all but those females receiving 333 mg/kg-day (group 2). Some marginal reductions in relative and absolute liver and kidney weights were observed among the treated groups, although it is unclear how much these changes were merely a consequence of dietary fluctuations.

A number of gross pathological findings were described in the report, although some were essentially sporadic in occurrence and, therefore, probably unrelated to dose. However, 4/6 high-dose females displayed smaller than normal spleens and enlarged adrenals, a feature that was also apparent in the single group-4 female that died prematurely. High-dose male rats displayed centrilobular degeneration of the

liver, while hepatocytic hyperplasia and increased cytoplasmic eosinophilia along with lymphocyte depletion in the thymus and spleen were noted in high-dose and other decedent females. However, the extent of these lesions in intermediate groups was not determined, an omission that did not allow a no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) based on any observations other than lethality to be established. Based on the findings in the study, these would be (nominally) 3000 and 9000 mg/kg-day, respectively.

A similar protocol to that described above was also used to determine the subacute toxicity of HMX in B6C3F1 mice (Greenhough and McDonald 1985b). Target dietary doses were, in males (groups 1–5), 0, 100, 300, 900 and 2700 mg/kg-day, and, in females (groups 1–5), 0, 320, 800, 2000 and 5000 mg/kg-day. As tabulated by the authors, the actual achieved average doses equivalent to these levels were, for male groups 1–3, 0, 119.5 and 383 mg/kg-day and 0, 344, 882.7 and 2045.6 mg/kg-day for female groups 1–4. The lethality rate for the full sequence of groups was 0/6, 0/6, 5/6, 6/6 and 6/6 in males and 0/6, 0/6, 2/6, 4/6 and 6/6 in females. All HMX-receiving groups displayed clinical signs in response to dosing that were marked by over-excitability in the lower dose groups and by a range of increasingly severe responses leading to death in the higher dose groups. Animals displayed an initial loss of weight that may have been associated with reduced food consumption. For the survivors, these parameters rebounded in parallel during the second week of exposure. This “recovery” was indicated also by similar terminal absolute and relative organ weights between treated and control groups. As described by the authors, the histopathological findings were characterized by a “dose-related increase” in hepatocellular hyperplasia and cytoplasmic eosinophilia, splenic red and white pulp, and thymic cellular depletion. However, by analogy to the 14-day study in rats (Greenhough and McDonald 1985a), the absence of any histopathological examinations of HMX-receiving survivors in the intermediate dose groups has forced the selection of a NOAEL and LOAEL based on lethality. These dose values were approximated as 100 and 300 mg/kg-day, respectively, based on the data for male mice, and to 800 and 2000 mg/kg-day, based on the data for female mice.

2.3.1.3 Mammalian Toxicity – Subchronic

As reported by Everett et al. (1985) and Everett and Maddock (1985), IRI carried out separate 13-week toxicological studies on HMX in F344 rats and B6C3F1 mice. Arising from the range-finding study in rats described earlier (Greenhough and McDonald 1985a), 20 rats/sex/group received dietary doses of 0, 50, 150, 450, 1350 and 4000 mg/kg-day (males) and 0, 50, 115, 270, 620 and 1500 mg/kg-day (females) (Everett et al. 1985). The actual achieved average doses equivalent to these target levels were, in males, 0, 51, 153.5, 461, 1394 and 4101 mg/kg-day, and 0, 50.3, 115.6, 273.3, 627.7 and 1511.9 mg/kg-day in females. In addition to a more extensive range of in-life, necropsy and histopathological observations

than in the 14-day study, all rats received an ophthalmic examination before dosing commenced and during week 13 of dosing. Clinical chemistry and hematological analyses were carried out on blood samples taken from the orbital sinus of 10 males and 10 females during weeks 5 and 12 of treatment. Four-hour urine samples were collected from a subset of subjects among the groups during weeks 5 and 12. These samples were monitored for glucose, blood, protein, ketones, color, pH, specific gravity, etc.

In contrast to the findings of the 14-day study in F344 rats (Greenhough and McDonald 1985a), there were no compound-related deaths and few clinical signs in evidence during the 13 weeks of dosing. All ophthalmological observations were unremarkable before and after treatment. However, body weight gain was reduced in a dose-dependent manner with varying degrees of statistical significance in some groups compared to controls. These changes may have been due, at least in part, to fluctuations in food consumption. Some potentially dose-related hematological changes were observed in both sexes of high-dose rats, including reductions in hemoglobin concentration, packed cell volume and erythrocyte count, and increases in methemoglobin levels. Sporadic, statistically significant differences in plasma enzyme activities (for example, in alkaline phosphatase) were observed in rats exposed to high-dose levels of HMX compared to controls. However, because the extent to which these changes were dose-dependent is uncertain, their relationship to HMX treatment cannot be unequivocally assigned. Although findings from gross necropsy were benign, some apparent dose-dependent histopathological changes were considered by the authors to be compound-related. These included the appearance of enlarged liver cells featuring large nuclei and granular eosinophilic cytoplasm with associated small necrotic foci, which were most evident in male rats.

The designation of a NOAEL for the histopathological effects of HMX in liver may be controversial. Thus, although the effects were most evident in males receiving the compound at the two highest doses, the IRIS compilers (USEPA 2000) and Talmage et al. (1999) chose a nominal dose level of 50 mg/kg-day as the NOAEL, based on an incidence of 2/19 in 150 mg/kg-day-receiving males compared to 0/20 in controls. However, since this difference is statistically insignificant by Fisher's exact test, a viable alternative choice of NOAEL might be the value of 150 mg/kg-day itself, an approach that appears to be more in line with the conclusions of the authors of the study (Everett et al. 1985). Regarding the issue of the precise value of the NOAEL, it could be argued that, if 150 mg/kg-day were adopted as the NOAEL, the next highest dose (450 mg/kg-day) would be unsatisfactorily high for the LOAEL, since the incidence of histopathological liver lesions was 20/20 at this level. Taking all of the incidence data together suggests that the subchronic points-of-departure (NOAEL and LOAEL) for the toxicological effects in F344 rats are likely to exist in a narrow dosimetric region between 100 and 400 mg/kg-day.

Other compound-related histopathological changes were evident in the kidneys of female F344 rats. The incidence of these lesions, characterized by focal atrophy and dilation of the tubules, achieved

statistical significance compared to controls at a dose level of 620 mg/kg-day and above (Fisher's exact test from the data in the study). These changes result in nominal NOAELs and LOAELs of 270 and 620 mg/kg-day, respectively, to protect against the kidney effects.

A 13-week study in B6C3F1 mice featured dietary administration of HMX at target dose levels of, in males, 0, 5, 12, 30, 75 and 200 mg/kg-day, and 0, 10, 30, 90, 250 and 750 mg/kg-day in females (Everett and Maddock 1985). The actual achieved average doses equivalent to these target levels were, in males, 0, 5.2, 12.2, 30.5, 75 and 199.8 mg/kg-day, and 0, 10.5, 30.8, 95.1, 257.1 and 784.5 mg/kg-day in females. A range of toxicological effects was observed similar to those in evidence in the rat study (Everett et al. 1985). However, in contrast to the findings in rats, the apparent toxicological consequences of the compound in the mice were profound, with 65% premature deaths observed in high-dose males and 100% deaths in high-dose females. Lower fatality rates were observed at lower dose levels supporting the conclusion that mortality was likely compound-related. However, other than lethality, few if any obvious HMX-related consequences were apparent among the survivors at any dose level, thereby rendering uncertain the causes of death among the high-dose animals and calling into question the utility of the study to delineate a sufficiently discriminating sub-threshold point of departure for the compound's toxicological consequences. Using mortality as the primary subchronic toxicological effect of HMX from the female mouse data, the nominal NOAEL would be 90 mg/kg-day, with a LOAEL of 250 mg/kg-day. These doses are strikingly similar to those identified for mortality in male B6C3F1 mice in the 14-day subacute toxicity study (Greenhough and McDonald 1985b).

2.3.1.4 Mammalian Oral Toxicity – Chronic

No experimental studies were identified that addressed the chronic toxicity of HMX.

2.3.1.5 Mammalian Oral Toxicity – Other

No other data relevant to oral exposures for mammals were found.

2.3.1.6 Studies Relevant for Mammalian TRV Development for Ingestion Exposures

The toxicological database on HMX is limited (Table 2). The toxicokinetic findings discussed by Wilson (1985) indicate that, typically, only a comparatively small proportion of an orally administered dose of HMX will be absorbed at the gastrointestinal barrier. In addition, Wilson (1985) pointed out that, in IRI experiments, only small amounts of the compound absorbed survived clearance in the urine, where the radioactivity partitioned mostly as highly polar metabolites. Therefore, since mammals clearly have the capacity to metabolize HMX, the fact that the radioactivity eliminated in the feces was overwhelmingly in the form of unchanged HMX supports the suggestion that this component of the load

probably represented unabsorbed substrate rather than HMX that had been absorbed and then undergone hepatobiliary recycling.

As with urinary metabolites, the little amount of compound deposited in the tissues will also have been changed to metabolites of HMX (Wilson 1985). This implies that the toxicological consequences of HMX, including the hepatic and renal changes seen in histopathological specimens and the compound-induced lethality evident at higher doses in either species of test animal (F344 rats and B6C3F1 mice), will probably have resulted from the biochemical activity of one or more metabolites of HMX rather than the parent compound. Unfortunately, examination of the carcasses of the high-dose mice receiving HMX for up to 13 weeks and the results of the necropsy and histopathological findings in animals treated at lower doses and surviving to term failed to offer any clues as to the causes of the premature deaths induced by HMX. In fact, there is little evidence of a single universally applicable mechanism by which HMX induces toxic effects leading to lethality in rodents. To the contrary, histopathological findings in F344 rats were inconsistent since, in the 13-week study (Everett et al. 1985), sublethal microscopic lesions in the liver were observed primarily in exposed males, while kidney effects were largely restricted to the females. This separate and gender-specific pattern of histopathological lesion formation argues against the existence of a single ubiquitous mechanism by which fatalities such as those observed in both sexes of mice from the 13-week study could have been induced.

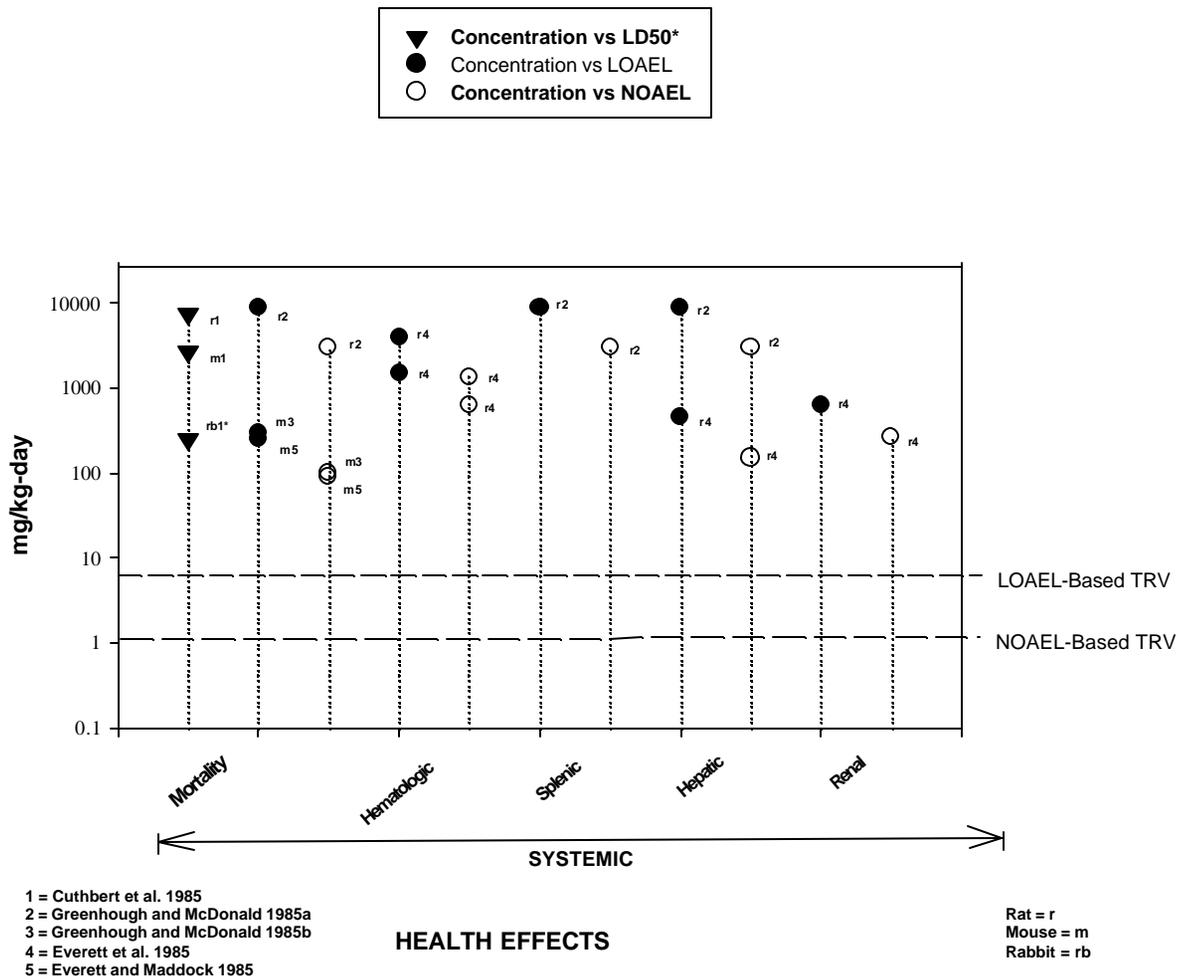
Table 2. Summary of Relevant Mammalian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
Cuthbert et al. (1985)	Rabbit	Single acute exposure	100 (m) ? (f)	250 (m) 50 (f)	Mortality, convulsions, miosis, mydriasis, slight hyperkinesias, labored respiration
Cuthbert et al. (1985)	Rat Mouse	LD ₅₀ LD ₅₀	7,360 (m&f) 2,710 (m&f)		
Greenhough and McDonald (1985a)	Rat (F344)	14-d	2981 (m) 1280 (f)	8504 (m) 3055 (f)	Lethality associated with histopathological liver lesions, lymphocyte depletion and spleen effects
Greenhough and McDonald (1985b)	Mice (B6C3F1)	14-d	120 (m) 883 (f)	383 (m) 2045 (f)	Lethality associated with histopathological liver lesions, lymphocyte depletion and spleen effects
Everett et al. (1985)	Rat (F344)	13-w	153 (m) 273 (f)	461 (m) 628 (f)	Histopathological lesions of the liver Focal atrophy and dilation of the kidney tubules
Everett and Maddock (1985)	Mice (B6C3F1)	13-w	95 (f)	257 (f)	Lethality

Taking the results of all the IRI studies on HMX together suggests that (unknown) metabolites formed from only a very small proportion of the administered load can induce lethality by an unknown mechanism, and that B6C3F1 mice are more susceptible to this effect than F344 rats. However, limited acute toxicity data suggest that the rabbit may be more susceptible to HMX than either B6C3F1 mice or F344 rats, a finding that permits the possibility that the subacute and subchronic NOAELs in rabbits might be even lower than those observed in B6C3F1 mice. Overall, the narrow range of animals employed in the experimental studies described and lack of any wildlife species data limits the confidence in the values selected as class-specific TRVs. These data are graphically presented in Figure 1.

Figure 1.

HMX HEALTH EFFECTS TO MAMMALS



* Indicates lowest lethal dose (LD_{Lo})

2.3.2 Mammalian Inhalation Toxicity

No inhalation studies conducted using animals were found.

2.3.3 Mammalian Dermal Toxicity

Cuthbert et al. (1985) reported data that included dermal toxicity evaluations in rats and rabbits. For rats, the dermal LD₅₀ was determined to be greater than 5.0 g/kg body weight. In rabbits, the percutaneous median lethal dose was determined to be 982.03 (861-1102) mg/kg in abraded/non-abraded skin tests for both sexes combined. HMX was found to be mildly irritating to the skin of rabbits although not an eye irritant. There was no evidence suggesting that HMX has sensitizing effects using the Magnusson-Kligman Maximisation Test in guinea pigs (Cuthbert et al. (1985).

2.3.4 Mammalian Toxicity – Other

Cuthbert et al. (1985) also conducted rat and rabbit intravenous studies using HMX using DMSO as a vehicle. Rat IV LD₅₀ was determined to be 25 and 38 mg/kg for males and females, respectively. Rabbit IV LD₅₀ was reported as between 10-15 mg/kg for both sexes.

2.4 Summary of Avian Toxicology

2.4.1 Avian Toxicity – Oral

2.4.1.1 Avian Oral Toxicity - Acute

An Approximate Lethal Dose (ALD) evaluation was conducted using 16 Northern Bobwhite (*Colinus virginianus*; Gogal et al. 2001). Birds were orally gavaged using a water vehicle at eight doses ranging from 125 to 2125 HMX mg/kg body weight. One bird of each sex was used for each dose group. There was only one death (female; 187 mg/kg) 6 days post exposure. There were no marked signs of overt toxicity.

A subsequent ALD was conducted using 8 birds, 4 groups, at doses ranging from 3188 to 10760 mg/kg. One female died at 7173 mg/kg that occurred 12 days post exposure. No dose related remarkable findings were attributed to exposure. The purity of the compounds was determined to be 98.5%. Additional ALDs were conducted where vehicle (e.g., corn oil) and fasting regime was evaluated, each with no predictable patterns in mortality. The authors report that crop contents consisted of impacted HMX in necropsied birds, suggesting the bolus effect from a non-absorbable substance. An on-going subchronic study confirms no adverse effects to birds from exposures as high as 10,000 ppm HMX in feed (Gogal pers. comm.), suggesting that HMX is largely not available for absorption.

2.4.1.2 Avian Oral Toxicity - Subchronic

No data are available.

2.4.1.3 Avian Oral Toxicity – Chronic

No data are available.

2.4.1.4 Avian Oral Toxicity – Other

No data are available.

2.4.2 Avian Inhalation Toxicity

No data are available.

2.4.3 Avian Dermal Toxicity

No data are available.

2.5 Summary of Amphibian Toxicology

Toxicological data for the effects of HMX in amphibian species was not located. Ecotoxicological research on the effects of this compound in amphibians is recommended.

2.6 Summary of Reptilian Toxicology

Toxicological data for the effects of HMX in reptilian species was not located. Ecotoxicological research on the effects of this compound in reptiles is recommended.

3. RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

The toxicity data for HMX are limited and variable. The acute toxicity information for HMX is limited to mice, rats, and rabbits. The data from the latter were generated from a weak experimental design (i.e., one rabbit of each sex for each dose group). The long-term (90-day and 13-week) oral data were developed using F344 rats and B6C3F1 mice. Mortality was a clearly relevant criterion that occurred in almost every study reviewed (Table 2.).

As outlined in USACHPPM Technical Guide 254, parameters used for derivation of a TRV should be ecologically relevant. Since mortality has a direct impact on the abundance of a particular species, this parameter has clear ecological relevance. All data concerning the toxicity of HMX has been generated from studies on laboratory animals. No wildlife species were used. Although very few animals were used, rabbits appear to be sensitive. Female rabbits died at every dose tested and exhibited symptoms consistent with animals in the high-dose group (Cuthbert et al. 1985).

Due to the limited data available, the approximation approach was used to derive the mammalian oral TRV for HMX (USACHPPM 2000). An uncertainty factor of 50 was used to derive the NOAEL-based approximate TRV from an acute LOAEL for mortality for female rabbits (Cuthbert et al. 1985). An uncertainty factor of 10 was used to derive the LOAEL-based approximate TRV from this same endpoint. These TRVs are consistent with the intravenous studies in rabbits and protective of male mortality that occurred at a far greater dose (250 mg/kg). Hence, these TRVs are consistent with these lines of evidence (Table 3). These TRVs were given a **Low** confidence rating since only one order was sufficiently characterized and there is evidence that suggests that the rodent data may not accurately characterize toxicity of HMX to other species of mammals.

Table 3. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose	Confidence
NOAEL-based	1 mg/kg/d	Low
LOAEL-based	5 mg/kg/d	Low

3.1.2 TRVs for Ingestion Exposures for Mammalian Foraging Guilds

Since the work conducted in rodents has been well documented, TRVs specific to mammalian omnivores could be derived. Using the information from Everett and Maddock (1985), mice appear to be more sensitive to the effects of oral HMX exposure than rats. They used a 13-week exposure regime where mortality was the only consistent endpoint, possibly due to the low oral bioavailability of HMX. These data are consistent with the findings of other work investigating acute, subchronic, and chronic exposures in rodents (Cuthbert et al. 1985, Everett et al. 1985, Greenhough and McDonald 1985a,b).

An uncertainty factor of 10 was used to derive the NOAEL-based approximate TRV from a subchronic NOAEL. An uncertainty factor of 4 was used to derive the LOAEL-based approximate TRV from a subchronic LOAEL. These TRVs are presented in Table 4. Given that these species have been

studied extensively, yet there are no other omnivore species evaluated, these TRVs are given a **Medium** confidence rating.

Table 4. Selected Ingestion TRVs for Mammalian Omnivores

TRV	Dose	Confidence
NOAEL-based	9 mg/kg/d	Medium
LOAEL-based	62.5 mg/kg/d	Medium

3.1.3 TRVs for Inhalation Exposures for the Class Mammalia

Not available at this time.

3.1.4 TRVs for Dermal Exposures for the Class Mammalia

Not available at this time.

3.2 Toxicity Reference Values for Birds

Not available at this time.

3.3 Toxicity Reference Values for Amphibians

Not available at this time.

3.4 Toxicity Reference Values for Reptiles

Not available at this time.

4. IMPORTANT RESEARCH NEEDS

The chemical/physical properties of HMX suggest that systemic exposure will be low for many organisms. The metabolic information from studies conducted in rodents show that most of the ingested HMX is excreted unchanged. However, the preliminary data for rabbits suggest differential absorption or biotransformation of HMX in herbivorous animals (e.g., ruminants, hindgut fermenters, etc.). Future work should focus on the possibility of these effects in herbivorous mammals. Additional data should be collected for reptiles and amphibians, though gastrointestinal exposure is likely to be less than that for mammals.

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APPENDIX A

LITERATURE REVIEW

The following files were searched in Dialog:

File 155 MEDLINE; File 156, TOXLINE, File 5 BIOSIS, File 10 AGRICOLA, File 203 AGRIS, File 399 Chemical Abstracts, File 337 CHEMTOX, File 77 Conference Papers Index, File 35 Dissertation Abstracts, File 40 ENVIRONMENTAL, File 68 Environmental Bibliography, File 76 Life Sciences Collection, File 41 Pollution Abstracts, File 336 RTECS, File 370 Science, File 143 Wilson Biological & Agricultural Index, File 185 Zoological Record, File 6 NTIS, File 50 CAB, File 144 PASCAL, File 34 SCISEARCH.

The search strategy for **Amphibians & Reptiles**:

- ◆ Chemical name, synonyms, CAS numbers
- ◆ AND (amphibi? or frog or frogs or salamander? or newt or newts or toad? or reptil? or crocodil? or alligator? or caiman? snake? or lizard? or turtle? or tortoise? or terrapin?)
- ◆ RD (reduce duplicates)

The search strategy for **Birds**:

- ◆ Chemical name, synonyms, CAS numbers
- ◆ AND chicken? or duck or duckling? or ducks or mallard? or quail? or (japanese()quail?) or coturnix or (gallus()domesticus) or platyrhyn? or anas or aves or avian or bird? or (song()bird?) or bobwhite? or (water()bird) or (water()fowl)
- ◆ RD

The search strategy for **Laboratory Mammals**:

- ◆ Chemical name, synonyms, CAS numbers
- ◆ AND (rat or rats or mice or mouse or hamster? or (guinea()pig?) or rabbit? or monkey?)
- ◆ AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- ◆ NOT (human? or culture? or subcutaneous or vitro or gene or inject? or tumo? or inhalation or carcin? or cancer?)/ti,de
- ◆ NOT ((meeting()poster) or (meeting()abstract))

- ◆ NOT (patient? or cohort? or worker? or child? or infant? or women or men or occupational)
- ◆ RD

The search strategy for **Wild Mammals**:

- ◆ Chemical name, synonyms, CAS numbers
- ◆ AND(didelphidae or opossum? or soricidae or shrew? or talpidae or armadillo? or dasypodidae or ochotonidae or leporidae) or canidae or ursidae or procyonidae or mustelidae or felidae or cat or cats or dog or dogs or bear or bears or weasel? or skunk? or marten or martens or badger? or ferret? or mink? Or aplodontidae or beaver? or sciuridae or geomyidae or heteromyidae or castoridae or equidae or suidae or dicotylidae or cervidae or antilocapridae or bovidae arvicolinae or myocastoridae or dipodidae or erethizontidae or sigmodon? or (harvest()mice) or (harvest()mouse) or microtus or peromyscus or reithrodontomys or onychomys or vole or voles or lemming?
- ◆ AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- ◆ RD

All abstracts from the DIALOG search were reviewed and encoded in ProCite. When the search retrieved an appreciable number of hits, *keywords in context* were reviewed to minimize costs before any abstracts were downloaded (Tier 1). However, when only a limited number of studies were identified by the search, the abstracts were downloaded at the time of the search (Tier 2).

As noted in Section 2.1, 32 hits on HMX were obtained in the initial search, all of which were selected for abstract evaluation. Seventeen of these articles and reviews were retrieved for this survey.