PUBLIC HEALTH GOALS FOR CHEMICALS IN DRINKING WATER

METHOXYCHLOR

September 2010

Governor of the State of California
Arnold Schwarzenegger

Secretary for Environmental Protection
California Environmental Protection Agency
Linda Adams

Director
Office of Environmental Health Hazard Assessment
Joan E. Denton, Ph.D.

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Methoxychlor
in Drinking Water

Prepared by

Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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LIST OF CONTRIBUTORS

PHG PROJECT MANAGEMENT	REPORT PREPARATION	SUPPORT
Project Director	***Author***	***Administrative Support***
Anna Fan, Ph.D.	Moira Sullivan, M.S.	Hermelinda Jimenez
	.	Janet Rennert
PHG Program Leader	***Primary Reviewers***	***Library Support***
Robert A. Howd, Ph.D.	John Budroe, Ph.D.	Charleen Kubota, M.L.S.
	Jim Donald, Ph.D.	
Comment Coordinator	***Final Reviewers***	***Web site Posting***
Michael Baes	Anna Fan, Ph.D.	Laurie Monserrat
	George Alexeeff, Ph.D.	
	Robert Howd, Ph.D.	

PREFACE

Drinking Water Public Health Goals
Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and adopt PHGs for contaminants in drinking water based exclusively on public health considerations. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances that may cause chronic disease shall be based solely on health effects and shall be set at levels that OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider potential adverse effects on members of subgroups that comprise a meaningful proportion of the population, including but not limited to infants, children, pregnant women, the elderly, and individuals with a history of serious illness.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. OEHHA shall consider additive effects of exposure to contaminants in media other than drinking water, including food and air, and the resulting body burden.
7. In risk assessments that involve infants and children, OEHHA shall specifically assess exposure patterns, special susceptibility, multiple contaminants with toxic mechanisms in common, and the interactions of such contaminants.

8. In cases of insufficient data for OEHHA to determine a level that creates no significant risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
9. In cases where scientific evidence demonstrates that a safe dose response threshold for a contaminant exists, then the PHG should be set at that threshold.
10. The PHG may be set at zero if necessary to satisfy the requirements listed above in items seven and eight.
11. PHGs adopted by OEHHA shall be reviewed at least once every five years and revised as necessary based on the availability of new scientific data.

PHGs are not regulatory requirements, but instead represent non-mandatory goals. Using the criteria described above, PHGs are developed for use by the California Department of Public Health (DPH) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Thus, PHGs are not developed as target levels for cleanup of ground or ambient surface water contamination, and may not be applicable for such purposes, given the regulatory mandates of other environmental programs.

Whereas PHGs are to be based solely on scientific and public health considerations, drinking water standards adopted by DPH are to consider economic factors and technical feasibility. Each primary drinking water standard adopted by DPH shall be set at a level that is as close as feasible to the corresponding PHG, with emphasis on the protection of public health. Each primary drinking standard adopted by DPH is required to be set at a level that is as close as feasible to the corresponding PHG, with emphasis on the protection of public health. MCLs established by DPH must be at least as stringent as the federal MCL, if one exists.

Additional information on PHGs can be obtained at the OEHHA web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL - METHOXYCHLOR IN DRINKING WATER

SUMMARY

The Office of Environmental Health Hazard Assessment (OEHHA) hereby establishes a Public Health Goal (PHG) of 0.09 micrograms per liter (μg/L) or parts per billion (ppb) for the pesticide methoxychlor (MXC) in drinking water. MXC can alter the normal functioning of the endocrine system; both the parent compound and its demethylated metabolites exhibit estrogenic and antiandrogenic activities. Although the reproductive system is a sensitive target of MXC toxicity in both males and females, oral exposure to MXC has also been shown to affect the liver, kidneys, immune, and nervous system in animals. The PHG is based on the lowest-observed-adverse-effect level (LOAEL) of 20 micrograms per kilogram body weight per day (μg/kg-day) of MXC given to pregnant mice (Judy *et al.*, 1999). A human drinking water consumption rate of 0.043 L/kg-day for pregnant adult females is used, with a relative source contribution of 20 percent from drinking water. A total uncertainty factor of 1,000 is used in the calculation, which is comprised of 10 for intraspecies differences, 10 for interspecies differences, and 10 for extrapolation from a LOAEL to a no-observed-adverse-effect level, or NOAEL. The effects observed at the LOAEL were significantly increased prostate weights (60 percent greater than controls) and decreased liver weights relative to controls in the male adult offspring.

This new PHG value of 0.09 μg/L is several orders of magnitude lower than the existing PHG of 30 μg/L set in 1999. This change reflects a number of new studies on the effects of exposure to low doses of MXC that have been published since the initial PHG value was developed.

The LOAEL used for derivation of this PHG is supported by similar LOAELs and NOAELs in other reproductive and developmental studies showing endocrine-disrupting effects at low levels following prenatal exposure (vom Saal *et al*., 1995; Palanza *et al*., 2002; Gioiosa *et al*., 2007). The child-specific reference dose (chRD) of MXC recently developed by OEHHA (OEHHA, 2005) is 0.02 μg/kg-day, based on the same study and approach used for this revised PHG. The U.S. Environmental Protection Agency (U.S. EPA) reference dose (RfD) for MXC is 5 μg/kg-day.

In vivo metabolism of MXC is relatively rapid, and it is not thought to bioaccumulate to the same degree as do many other halogenated hydrocarbon pesticides. MXC is also only moderately persistent in the environment, and is rarely found in air, soil, or water except near sites of production or disposal. California suspended pesticidal usage of MXC in 1995, and U.S. EPA suspended all its product registrations in 2000 and revoked all MXC tolerances for residues in food in 2002. For this reason, significant human exposure to MXC is not anticipated, although MXC residues may still be found in food grown in other countries.

Cancer bioassays of MXC have been negative. Sensitive populations, sensitive developmental periods, and exposure to other chemicals with estrogenic activity have been considered in calculating the health protective concentration for MXC in drinking water.

The U.S. EPA Maximum Contaminant Level (MCL) and Maximum Contaminant Level Goal (MCLG) for MXC in drinking water are both 0.04 mg/L (40 μg/L or ppb) (U.S. EPA, 2009), and were originally finalized in 1991 (56 FR 3526, 01/30/91). The California MCL is 0.03 mg/L (30 μg/L), which was decreased from 0.04 mg/L in 2003 in response to the 1999 PHG of 0.03 mg/L (30 μg/L) (DPH, 2009).

INTRODUCTION

The purpose of this document is to describe the development of a revised PHG for the insecticide methoxychlor in drinking water. This DDT analog was very heavily used after cancellation of DDT in the 1970s. In the early '90s about 300,000 to 500,000 pounds of MXC were used per year in the U.S. (ATSDR, 1994). Compared to DDT, MXC is rapidly metabolized both in the environment and in living organisms, and so was not thought to produce the long-lasting toxicity and bioaccumulation which led to the cancellation of DDT. However, MXC has been shown to cause persistent impairment of the reproductive tract and other organ systems that mature under the influence of gonadal hormones following early exposure to low, environmentally relevant doses.

Use of MXC was suspended in California in late 1995 due to deficiencies in its toxicity study database. MXC use in California has almost completely ceased, with only 6 pounds of active ingredient reported used in agriculture in 2007 (DPR, 2007). In January 2000, U.S. EPA canceled all product registrations of MXC, and in July 2002 revoked all tolerances for residues of this pesticide in food due to significant concerns about the effects of MXC on human health and the environment (U.S. EPA, 2004a). MXC was used by the U.S. and the Organization for Economic Cooperation and Development (OECD) as one of the key chemicals in validating components of the Endocrine Disruption Screening Program.

The potential for detecting MXC in California drinking water has undoubtedly decreased greatly since its suspension**.** Prior to cancellation, MXC had been approved for use against insects on fruit and shade trees, vegetables, dairy and beef cattle, in home gardens, commercial greenhouses, in landscape maintenance, in food storage and seed pretreatment, and in public health insect control. It was often formulated with other pesticides in products.

An MCL of 0.03 mg/L was established by the California Department of Health Services (DHS; now Department of Public Health, DPH) in 2003, in response to the 1999 PHG of 0.03 mg/L (DPH, 2009). The federal MCL for MXC is set at 0.04 mg/L (U.S. EPA, 2009). OEHHA considered MXC to be a chemical of concern for school site risk assessment, and therefore developed a child-specific reference dose (chRD) of MXC, which is 0.02 μg/kg-day (OEHHA, 2005). MXC is not listed under California's Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65) as a chemical

known to the state to cause cancer or reproductive toxicity. U.S. EPA has judged MXC as class D, "not classified as to human carcinogenicity" (U.S. EPA, 2010).

In this document, available data on the toxicity of MXC are evaluated, particularly in the context of recent perspectives on environmental estrogens. The U.S. EPA's reproductive toxicity risk assessment guidelines (U.S. EPA, 1996) were also considered. To determine a public health-protective level of MXC in drinking water, relevant studies were identified, reviewed and evaluated, and sensitive developmental periods and human subpopulations have been considered.

CHEMICAL PROFILE

Chemical Identity

MXC, chemical name 2,2-bis(*p*-methoxyphenyl)-1,1,1-trichloroethane, is a bicyclic aromatic chemical related to DDT. The chemical formula is $C_{16}H_{15}Cl_3O_2$; its structure is shown in Figure 1 below.

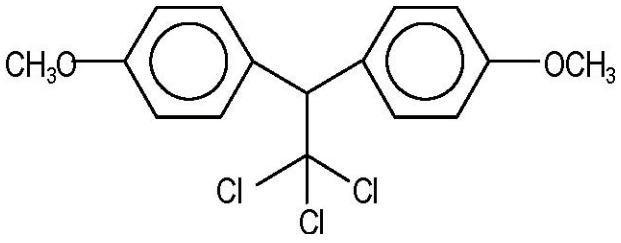

Figure 1. Chemical structure of methoxychlor

MXC was first synthesized in 1893, and commercial production in the U.S. began in 1946. It was initially approved for use as an insecticide on numerous agricultural crops as well as on cattle, goats, sheep and swine. Trade names include Maralate, Marlate, and Metox. Its CAS number is 72-43-5.

In the synthesis of this chemical, many impurities were produced. Earlier versions were only about 50 percent pure; more recently, the technical grade was improved to about 88-90 percent purity (ATSDR, 1994). Some of the congeners may have greater toxicity and environmental persistence than MXC.

Physical and Chemical Properties

Important physical and chemical properties of MXC are shown in Table 1. Like other halogenated aromatics, MXC is lipophilic, only slightly soluble in water and is poorly volatile. It binds rather tightly to soil. The slow volatilization and distribution around the globe that has been documented for other halogenated hydrocarbons (Wania and Mackay,

1996) is less of a problem for MXC because of its short environmental half-life (see Environmental Occurrence and Human Exposure).

Table 1. Physical and Chemical Properties of Methoxychlor (ATSDR, 2002)

Property	**Value**
Molecular weight	345.65
Color	Pale yellow
Physical state	Crystalline solid
Odor	Slightly fruity or musty
Odor threshold (water)	4.7 ppm
Melting point	89^{o}C (pure), 77^{o}C (technical grade)
Boiling point	Decomposes
Solubility Water Organic solvents	 0.045 mg/L at 25^{o}C Soluble in aromatics, ketones, aliphatics, alcohols
Density	1.41 g/cm^{3}
Partition coefficients Log K_{ow} Log K_{oc}	 4.7-5.1 4.9
Vapor pressure (25^{o}C)	1.4 x 10^{-6} mm Hg (est.)
Henry's law constant	1.6 x 10^{-5} atm-m^{3}/mol (est.)
Conversion factors	1 ppm = 14.14 mg/m^{3}

Production and Uses

This organohalogenated pesticide was heavily used following the cancellation of DDT in the 1970s, with peak U.S. production in the late 1970s to early 1980s of over 5 million pounds. In 1986, usage was estimated to be about 600,000 pounds, and about 300,000 to 400,000 pounds per year in 1990 to 1991; Kincaid Enterprises was listed as the sole producer and distributor of MXC in the U.S. (ATSDR, 2002). Suspension of MXC use in California (December 26, 1995) has resulted in a significant decline in production and use. In January 2000, U.S. EPA canceled all product registrations of MXC. The annual pesticide use report published by the California Department of Pesticide Regulation (CDPR) shows only 6 pounds of active ingredient used in 2007, the last year for which information is available (DPR, 2007). Table 2, below, provides data on MXC use for the years 1997-2007 (DPR, 2007), as its supplies were used up.

Table 2. Methoxychlor Pesticide Use in California, 1997-2007.

	1997	1998	1999	2000	2001+	2002	2003	2004	2005*	2006	2007
Gross Pounds Pesticide Applied	358	566	16	26	41	144	3	1	13	130	6
Cumulative Acres Treated	131	194	140	197	88	24	0	44	26	395	43

Adapted from DPR (2007).
+ Complete 2001 data for Kern County were never submitted. The missing data include approximately 32,000 records totaling roughly 10 million pounds of pesticides.
*The 2005 data for Ventura County are incomplete because not all of the data were available at the time of DPR's release. An estimate from 2002-2004 suggests that the 2005 total pounds (for all pesticide use) is underreported by approximately 500,000 pounds.

The potential for detectable concentrations of MXC in California drinking water has undoubtedly decreased greatly since its uses were suspended, making it no longer a human health concern. MXC was registered by the U.S. EPA, either alone or in combination with other pesticides, for use against houseflies, mosquitoes, cockroaches, chiggers, various arthropods found on field crops, and insect pests in stored grain or seed for planting. It was registered for use on more than 85 crops, including fruits, vegetables, soybeans, nuts, and alfalfa. MXC was also approved for use on forests, ornamental plants, and for insect control around houses, barns, and other agricultural premises (ATSDR, 1994). It was often formulated with other pesticide products, such as captan, diazinon, and malathion. MXC was available in many forms, including technical-grade concentrate, wettable powders, dusts, granules, emulsifiable concentrates, and pressurized sprays for home use.

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Air

Most of the introduction of MXC to air would occur during and after its use as a pesticide. Because it has a low vapor pressure and binds well to soil, air concentrations would be expected to be quite low except in the immediate area of a spray application. The photo-oxidation half-life of MXC in air is not known, but it has been estimated at 1-11 hours (Howard, 1991). Some long-distance transport of MXC in air, perhaps bound to fine particles, is indicated by the detection of MXC in arctic snow (Welch *et al*., 1991). However, the lower stability of MXC compared to other organochlorinated pesticides makes it less likely to become a global problem.

A survey of pesticide levels in air in two U.S. cities found mean levels of MXC of 0 to 100 picogram/m^3 in outdoor air and 200 to 300 pg/m^3 in indoor air (U.S. EPA, 1990a).

In a Canadian study, the yearly mean level of MXC in air was reported to be 1.7 pg/m^3. Levels were higher during insect control periods (up to 27 pg/m^3) and generally below the detection limit (about 0.1 pg/m^3) during non-use time periods (Hoff *et al*., 1992). Levels of MXC in California air would thus be expected to be extremely low.

Soil

MXC is degradable in soil to less hydrophobic compounds, both under aerobic and anaerobic conditions. Anaerobic biodegradation half-life was reported to be less than 30 days, while aerobic biodegradation half-life was greater than 100 days (Muir and Yarechewski, 1984). Residues were detectable in soil at least 18 months after soil treatment (Golovleva *et al*., 1984). The major environmental degradation pathways involved dechlorination and demethylation. The extent to which the degradation products may accumulate in soil is not clear, although some bacterial strains can extensively metabolize the pesticide. It should be noted that the demethylated products have estrogenic activity (Cummins, 1997). Intact MXC binds tightly to soil, and will be found in the top few inches after agricultural applications. The metabolites, being more polar, can migrate in soil (Golovleva *et al*., 1984). Migration of MXC bound to sediment particles is also possible.

Water

MXC has been occasionally detected in surface waters at low levels, ranging from 0.032 to 15 nanograms/L (ng/L) (ATSDR, 1994). It was not found, however, in domestic or municipal drinking water supplies in several surveys in various regions of the country (U.S. EPA, 1990a,b). MXC has been found in surface waters near points of application for pest control, and in 19 groundwater and 7 surface water samples collected near waste disposal sites (ATSDR, 1994, 2002). MXC has not been found in water samples from agricultural wells in recent surveys by the California Department of Pesticide Regulation (Troiano *et al.*, 2001).

MXC in surface waters would be distributed mostly in the sediment fraction due to its low water solubility and tight binding to lipophilic sites on soil particles. Overall, water would be expected to be a minor source of exposure to MXC for California residents.

Food

Residues of MXC have been found in a small proportion of food samples of various types, including vegetables, fruits, and grains. Bioconcentration can occur, dependent on the rate of metabolism of MXC. Bioconcentration factors of about 100 to 8,000 have been reported in several fish species. Samples of fish from the Great Lakes occasionally had detectable MXC, with some tissue levels as high as 100 ppb (ATSDR, 1994). In the U.S. FDA's Total Diet Study in 1995, MXC was detected at low levels in less than 3 percent of the samples (which was below the cutoff point for specific discussion of incidence and levels) (U.S. FDA, 1996). MXC was not reported present in produce sampled in the 2007 and 2008 California pesticide monitoring program (DPR, 2009).

MXC can be excreted in a biologically active form in milk, which could be relevant for infants and children (Ivey *et al*., 1983; Appel and Eroschenko, 1992; Chapin *et al*., 1997). Average daily intake values for MXC in food were calculated as 0.001 to 0.008 μg/kg-day by Gunderson (1988) based on the FDA's monitoring for the total diet study in 1982-1984. Infants' daily dietary intake was estimated to average 0.019 μg/kg-day from the FDA's 1980-1982 data. Current exposures are expected to be much lower, based on the substantial decrease in agricultural usage and its moderate environmental persistence. However, it is reasonable to conclude that foods, including fish, would be the major MXC exposure source for California residents.

U.S. EPA revoked all tolerances for residues of MXC in food in July 2002. Food containing residues of this pesticide are considered to be unsafe and therefore adulterated under the Federal Food, Drug and Cosmetic Act (FFDCA). Such food may not be distributed in interstate commerce.

Studies in animals have shown combination effects (potentiation) of MXC and genistein (a phytoestrogen naturally occurring in soybeans) on reproductive development of offspring of both sexes (You *et al*., 2002; Wang *et al.,* 2006). Dietary exposure to phytoestrogens is common for both animals and humans; exposures occur through regular dietary intake or through nutritional supplements of phytoestrogens. Phytoestrogens such as genistein have the potential to modulate biological responses to other endocrine-active compounds, and are themselves capable of causing developmental alterations in animal studies at concentrations normally found in animal food (Casanova *et al.,* 1999).

Other Sources

There should be no significant occupational exposures to MXC, since the pesticide has been suspended from use. Similarly, exposures during home use in gardens and orchards, as well as for flea and insect control, should have decreased as home supplies were used up. The major exposure source for California residents should now be MXC in food, from commodities grown outside the U.S.

METABOLISM AND PHARMACOKINETICS

Absorption

Oral absorption of MXC is apparently quite efficient, although no specific estimates are available for humans. Oral absorption in mice has been estimated to exceed 90 percent (ATSDR, 1994). For this assessment, oral absorption is assumed to be equivalent in humans and experimental animals. Dermal absorption of MXC deposited directly on skin would be expected to be low and slow. Limited studies in goats and cows have demonstrated a low degree of systemic absorption after dermal applications (Skaare *et al*., 1982; Davison *et al*., 1983; Ivey *et al*., 1983). Absorption should be similar to DDT, for which dermal penetration has been measured as 9 to 30 percent in rhesus monkeys after the pesticide was applied in acetone (Wester *et al*., 1990). Data on inhalation

absorption of MXC are not available. We assume that pulmonary absorption of MXC would be essentially complete, *i.e.*, equivalent to the inspired volume minus dead space, or about 70 percent in humans.

Distribution

MXC is distributed throughout the body, and because of its lipophilicity is taken up very well into fat. Having a log K_{ow} of 4.7 to 5.1, at equilibrium it would be about 100,000 times higher concentration in a lipid phase than in a contiguous aqueous phase. In actual tissues the partitioning is not this extreme because of lipids in blood and water in fatty tissues. With repeated daily administration, fat and liver levels reach an equilibrium, then appear to decrease, possibly because of induction of metabolism. Residual levels decline rapidly after feeding stops, so that MXC levels approach the detection limit within a few days (ATSDR, 1994). MXC fed to rats in the diet for 2 years at levels of 25, 200, or 1600 mg/kg was found in fat, kidney, liver and brain tissue (Hodge *et al*., 1952). MXC crosses the blood-brain barrier and the placenta, and also partitions into the lipids of milk (Ivey *et al*., 1983; Swartz and Corkern, 1992; Appel and Eroschenko, 1992; Cummings, 1997; U.S. EPA, 1998). The metabolites are secreted into the bile. The extent of enterohepatic circulation is unknown, but the metabolites are mainly excreted in the feces.

Metabolism

MXC is metabolized by cytochrome P450 isozymes in liver in both rodents and humans, to produce mono- and bis-hydroxy O-demethylated (phenolic) metabolites (Li *et al.*, 1995; Dehal and Kupfer, 1994; Kupfer *et al*., 1990). This is an NADPH-requiring, phenobarbital-inducible reaction. Dehydrochlorination occurs concurrently, so that a mixture of demethylated, dehydrochlorinated products is formed. These major mammalian metabolic pathways are summarized in Figure 2. Subsequent metabolic reactions may include ring hydroxylation in the meta positions, complete dechlorination, and various conjugation reactions to form more hydrophilic products. The metabolic products are secreted in the bile, presumably in the form of conjugates, and are ultimately excreted in feces. The actual mixture of reaction products found *in vivo* (and its net estrogenic activity) is complicated by the presence of several different impurities in the technical-grade MXC (Kupfer and Bulger, 1987).

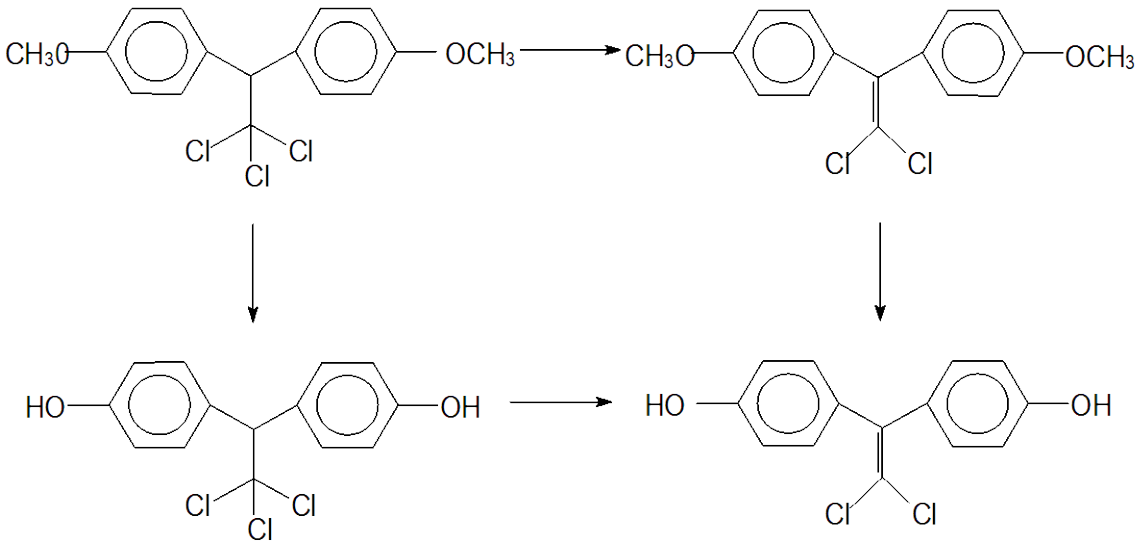

Figure 2. Major *in vivo* metabolic pathways for methoxychlor

Excretion

MXC was about 90 percent excreted into the feces in the form of metabolites in mice; the other 10 percent was excreted in the urine (Kapoor *et al*., 1970). In a lactating female, however, a fraction of the total intake is secreted into milk in the form of both intact MXC and phenolic metabolites. For highly lipophilic chemicals with very long half-lives, incorporation into milk can represent a significant excretion pathway, and a correspondingly high exposure pathway for babies drinking the milk. This has much less significance for MXC because it is rapidly lost from the body by other pathways.

Because the liver O-demethylation reactions are relatively rapid, MXC does not accumulate in the body like its analog, DDT (p,p'-dichlorodiphenyltrichloroethane). The more water-soluble phenolic metabolites of MXC are readily metabolized further and excreted. In addition, the inducibility of the cytochrome P450 metabolizing enzymes at higher doses of MXC should be considered. With induction of metabolism, any chronic toxic effects due to the intact chemical should be lessened, while estrogenic effects, which are largely due to the phenolic metabolites, could be enhanced.

TOXICOLOGY

Toxicological Effects in Animals

Mechanism Studies

MXC can alter the normal functioning of the endocrine system and is classed as an endocrine-disrupting chemical. Both estrogenic and antiandrogenic activities can be observed, mostly mediated by the demethylated metabolites of MXC rather than the parent compound (Maness *et al*., 1998; Gaido *et al*., 2000). The two major phenolic metabolites of methoxychlor, 2-(*p*-hydroxyphenyl)-2-(*p*-methoxyphenyl)-1,1,1-trichloroethane and 2,2-*bis*-(*p*-hydroxyphenyl)-1,1,1-trichlorethane (HPTE), have been shown to have a higher affinity for the estrogen receptor than the parent compound; of the two, the most active methoxychlor metabolite has been shown to be HPTE, a DDT analog (Bulger *et al.*, 1985). Studies have shown that HPTE inhibits the binding of ^{3}H-E_2 to the estrogen receptor (Ousterhout *et al.*, 1979), exerts agonist activity at estrogen receptor α (ERα), and antagonist activity at estrogen receptor ß (ERß) and the androgen receptor (Gaido *et al*., 1999, 2000). The mechanism of action of HPTE appears similar to the endogenous estrogenic compound 17ß-estradiol. HPTE, like 17ß-estradiol, decreases cytosolic estrogen receptors and elevates nuclear estrogen receptors (Kupfer and Bulger, 1979). A number of studies have shown a parallel biological mechanism between estradiol and MXC (HTPE) (Cummings and Metcalf, 1994; Cummings and Metcalf, 1995; Metcalf *et al.*, 1995, vom Saal *et al.*, 1995; Judy *et al.*, 1999), or provided perspective on the significance of the interactions with estrogenic receptors (Alworth *et al.*, 2002; Welshons *et al.*, 2003). A dosage of 500 mg MXC/kg increased uterine epidermal growth factor (EGF) receptor binding activity by 156 percent over endogenous levels after 12 hours in immature female Sprague-Dawley rats; a dosage of 20 µg 17ß-estradiol led to a 175 percent increase in uterine EGF over untreated levels after 12 hours (Metcalf *et al.*, 1996). EGF and its receptor (EGF-R) have been implicated as mediators for estrogen-induced cellular growth, and possible mediators of uterine function (Nelson *et al.*, 1991).

MXC is known to induce liver cytochrome P4502B and P4503A enzymes in the rat (Li *et al*., 1995). Liver also contains estrogen receptors. Exogenous estrogens are known to affect the metabolic activity of the liver (von Schoultz *et al*., 1989). Hence, exposure to environmental estrogens such as MXC might result in an alteration in the number of hepatic estrogen receptors, thereby affecting hepatic metabolism of estrogen.

Acute Toxicity

High doses of MXC cause tremors, convulsions, and other signs of neurological stimulation. These acute effects are similar to those of DDT and the Type 1 pyrethroids. Acute oral LD_{50}s are generally greater than 3,000 mg/kg in mammals and 2,000 mg/kg in birds for technical-grade MXC. More purified preparations tend to be less toxic, with rat LD_{50}s greater than 5 g/kg (Cummings, 1997). Hodge *et al.* (1950) determined the 72-hour LD_{50} in rats to be 5 g/kg for the technical grade of MXC. The purified material was

found to be somewhat less toxic; at 72 hours, a mortality of ten percent was observed at 5.8 g/kg. Histopathological evaluation showed a significant reduction in testes weight and atrophic changes in sections of the treated testes. This level of toxicity is considerably less than that of DDT (LD_{50} = 0.25 g/kg). However, fish and aquatic invertebrates are quite sensitive to MXC. The 96-hr LC_{50}s for fresh and salt-water fish range from about 0.006 to 0.1 ppm, while the LC_{50}s for various aquatic invertebrates are as low as 0.001 ppm (Sax, 1987; U.S. EPA, 1988).

Subchronic Toxicity

With repeated dosing, reproductive effects become apparent in both males and females. These appear to be mediated largely by the phenolic metabolites of MXC, which bind much more efficiently to estrogen receptors than MXC does (Cummings, 1997; Bulger *et al.*, 1985). These effects are discussed below. However, it is not clear whether all the subchronic effects of MXC are mediated through direct actions on reproductive organs. Effects on the hypothalamic-pituitary axis of male rats have also been noted at moderate doses (25 and 50 mg/kg-day for 56 days), which may be a direct effect rather than mediated through a testicular feedback loop (Goldman *et al*., 1986). MXC may also alter some hormonal systems through alteration of liver metabolism, such as metabolism of thyroid hormones, although it does not appear to be a strong liver enzyme inducer (Zhou *et al*., 1995).

Genetic Toxicity

MXC has been found to be negative in several Ames assay mutagenicity tests (Simmon, 1979; Probst *et al*., 1981; Waters *et al*., 1982) with and without metabolic activation. Unscheduled DNA synthesis assays in rat hepatocytes and human fibroblasts were also negative, as was the Drosophila sex-linked recessive lethal assay (Simmon, 1979; Probst *et al*., 1981; Waters *et al*., 1982). Negative results have been reported in a transformation assay in rat embryo cells (Dunkel *et al*., 1981; Traul *et al*., 1981), in Syrian hamster embryos (Dunkel *et al*., 1981), and in Chinese hamster ovary cells (Oberly *et al*., 1993). A positive result has, however, recently been reported for the induction of forward mutations in the mouse lymphoma assay (Oberly *et al*., 1993). In addition, positive results have been reported in the mouse lymphoma cell mutagenesis assay (Mitchell *et al*., 1988; Myhr and Caspary, 1988).

Formation of protein adducts has been reported in methoxychlor metabolism studies in rat liver. These covalently bound liver microsomal protein adducts appear to be formed in the cytochrome P450-mediated metabolism of MXC, although the mechanism and significance is not entirely clear (Bulger *et al*., 1983; Bulger and Kupfer, 1989, 1990). Formation of DNA adducts has not been reported. MXC does not induce DNA breaks in human or rat testicular cells in an *in vitro* DNA-damage assay (Bjorge *et al*., 1996).

Developmental and Reproductive Toxicity

Reproductive effects of MXC have been extensively studied in animals. The reproductive system is a sensitive target of methoxychlor toxicity in both males and females. Effects include histopathological changes in the reproductive organs and accessory glands, disrupted sexual maturation and reproductive function, altered hormone levels, and changes in a wide variety of endocrine-related parameters, such as sexual behaviors. These effects result largely from the estrogenic activity of both the O-demethylated metabolites of methoxychlor and some of the O-demethylated contaminants of technical grade methoxychlor; intact MXC has a lesser affinity for the estrogen receptors (Bulger *et al*., 1985; Cummings, 1997). MXC is not listed as a reproductive toxicant under California's Proposition 65. Information regarding the reproductive effects of MXC in male and female animals is presented separately below. Some additional perspective on developmental or reproductive effects after long-term exposures is provided in the chronic toxicity section.

Reproductive Effects in Female Animals

MXC affects development of the female reproductive system. The most sensitive effects have been observed with perinatal treatments. This is presumably a time of high sensitivity because of rapid development of the reproductive system during this period. Table 3 summarizes *in vivo* effects of MXC in females of different mammalian species.

Table 3. *In vivo* Effects of Methoxychlor on Females of Several Mammalian Species

Parameter	Species	Effects	Reference
Folliculogenesis	Rat	Follicular development↓, follicular Atresia↓, AMH in ovary↑	Bal, 1984; Uzumcu *et al.,* 2006
	Mouse	Follicular atresia↑, antral follicle growth↓, persistent vaginal estrus, lipid accumulation in ovarian cells↑, ROS in antral follicles↑	Martinez and Swartz, 1991, 1992; Eroschenko *et al.,* 1995; Borgeest *et al.,* 2002, 2004; Golub *et al.,* 2003; Gupta *et al.,* 2006; Miller *et al.,* 2006
	Rhesus Monkey	Shorter follicular stages	Uzumcu and Zachow, 2007
Ovulation	Mouse	Ovulation rate↓, irregular estrous cycle	Chapin *et al.,* 1997; Eroschenko *et al.,* 1997; Suzuki *et al.,* 2004
Implantation / pregnancy	Rat	Embryo implantation↓	Cummings and Laskey, 1993
Estrogenic Response	Mouse	Uterine weight↑	Eroschenko, 1991; Walters *et al.,* 1993; Swartz *et al.,* 1994
	Rat	Vaginal opening↑, cornification↑, UW↓, uterus+ vagina weight↑, uterine peroxidase↑, ornithine	Eroschenko, 1991; Cummings and Metcalf, 1994

Parameter	Species	Effects	Reference
		decarboxylase↑	
Uterine gene	Mouse	Hox 10 gene expression↓	Fei *et al.*, 2005
	Rat	Calbindin-D_{9k} mRNA↑	Shin *et al.*, 2007
Estrogen receptor (ER)	Mouse	ER gene in neonatal uterine epithelium↑	Eroschenko *et al.*, 1996
Embryo Development	Rat	Preimplantation embryonic loss	Cummings and Perreault, 1990; Hall *et al.*, 1997

Adapted from Tiemann (2008). ↓ = decrease; ↑ = increase

Chapin *et al*. (1997) gavaged Tac:N(SD)fBR rats with 95 percent MXC in corn oil from gestational day (GD) 7 to postnatal day (PND) 7 at doses of 0, 5, 50, or 150 mg/kg-day. The pups were then individually dosed by gavage from PND 7 to day 42. One set of offspring was allowed to mate at 12 weeks of age and the females were killed for examination at gestational day 18. Extensive gross, histopathological, chemical, and behavioral measurements were made on both male and female treated offspring. Adverse effects were seen on multiple reproductive system parameters in both sexes (effects on male offspring are discussed under the male reproductive section of this document). The most significant low-dose effects were seen in females, including LOAELs of 5 mg/kg, the lowest dose tested, for delayed vaginal opening (VO) and decreased ovary weight at PND 46. Decreased weight of the empty uterus at day 18 of pregnancy and lowered follicle-stimulating hormone (FSH) levels during estrus were also observed in adult female rats after the perinatal treatments with MXC at 5 mg/kg-day and higher. These effects are tabulated in Table 4.

Table 4. The Critical Sensitive Effects of Methoxychlor in Female Rats (Chapin *et al.*, 1997)

EFFECT	DOSE, mg/kg-day			
	Control	5	50	150
Age at vaginal opening (days ±SE)	37.4±0.6	35.2±0.5*	30.8±0.2*	33.4±0.3*
Decreased ovary weight at postnatal day 46 (g ±SE, % of control)	0.065±0.004 (100%)	0.047±0.003* (72%)	0.030±0.043* (46%)	0.03±0.008* (46%)
Weight of empty uterus at gestation day 18 (g ±SE, % of control)	5.13±0.20 (100%)	4.06±0.30* (79%)	2.51±0.60* (49%)	None pregnant
Serum FSH levels during estrus (log of ng/ml ±SE, % control)	0.79±0.03 (100%)	0.57±0.05* (72%)	0.33±0.04* (42%)	NR

*p <0.05; NR = not reported

Figure 3 shows the effects of MXC on ovary weight at PND 46 after the perinatal treatments to dam and offspring, plotted with dose on a logarithmic scale to show the log-linear extrapolation of effect to low levels. On this scale, the effect would appear to extrapolate to a 10 percent decrease from control levels (a common level for benchmark extrapolation methods) at about 1 mg/kg-day. The zero effect level (ovary weights of 0.065 g) would correspond to about 0.5 mg/kg.

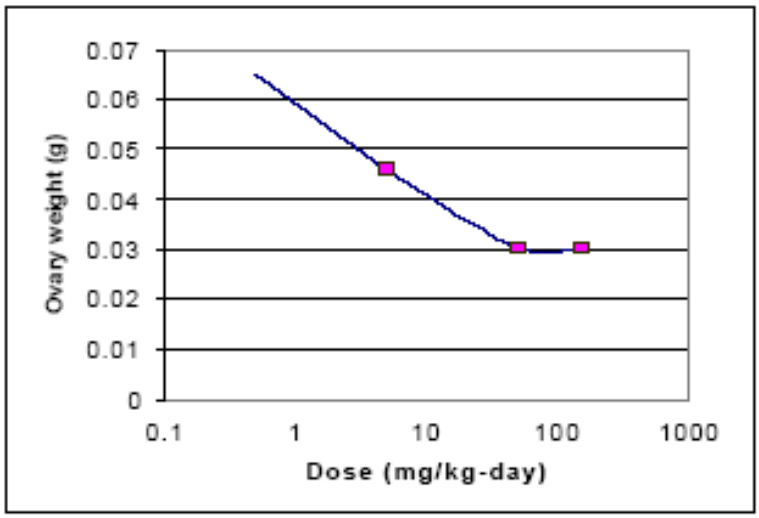

Figure 3. Effect of methoxychlor on ovary weight (Chapin *et al*., 1997)

Figure 4 shows the weights of the empty uteri on day 18 of pregnancy and the FSH levels during estrus in adult female rats perinatally treated with MXC. These values are plotted using a linear dose-response function. It could be considered that the effects at 50 mg/kg-day (the largest dose plotted) are near the maxima for these functions because estrous cycles were interrupted at the next higher dose (150 mg/kg-day), and none of the females became pregnant. When plotted on a logarithmic dose scale, a straight-line extrapolation through 5 and 50 mg/kg-day passes through zero effect at 0.5 to 1 mg/kg-day.

The male rats showed less sensitivity to MXC than the females. Only one measured parameter, the seminal vesicle weight, was significantly decreased at 5 mg/kg-day, and this did not exhibit a dose-response function (no significant effect at 50 mg/kg). Females receiving the highest-dose perinatal treatment of 150 mg/kg-day failed to produce litters when mated as adults (0/15), while at the 50 mg/kg-day dose, only 3/15 delivered live litters. In summary, the Chapin *et al*. (1997) data appear to show a sensitive effect on reproductive parameters with a LOAEL of 5 mg/kg-day.

The day of VO and first estrus was also observed to be significantly earlier in female rats exposed to 25-100 mg/kg-day of MXC beginning on post-partum day 21 (Gray *et al*., 1989). Estrous cyclicity started earlier in animals exposed to 25 mg/kg-day, at a normal age in animals exposed to 50-100 mg/kg-day, and was delayed in animals dosed with 200 mg/kg-day. Similarly, precocious VO and estrus were noted in female rats exposed in utero, through their mother's milk, and/or postweaning to 50-60 mg/kg-day (Harris *et al*., 1974; Gray *et al*., 1989).

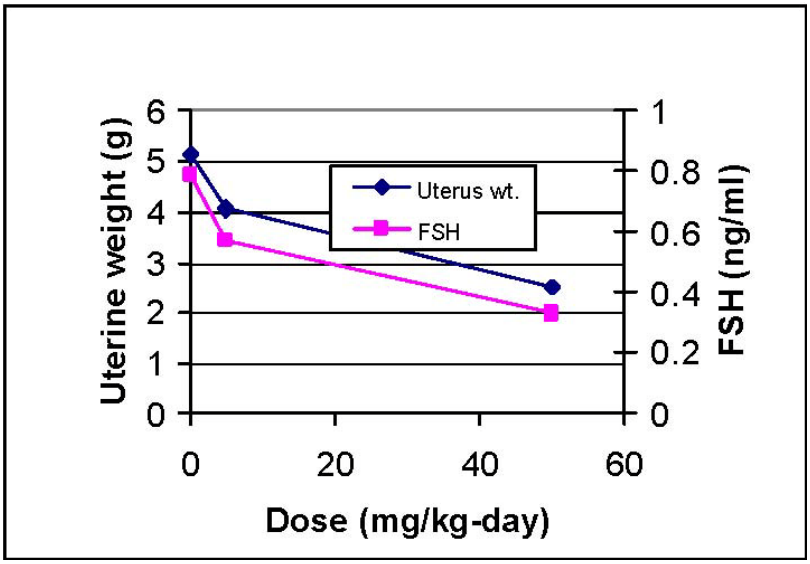

Figure 4. Weight of pregnant uterus and FSH levels during estrus (Chapin *et al*., 1997)

MXC produces gross and histopathological changes in female mouse reproductive tissues after repeated oral treatments. Lipid accumulation was observed in ovarian interstitial and thecal cells of mice exposed to 200 mg/kg-day for four weeks (Martinez and Swartz, 1992). A two- to three-fold increase in uterine weight was observed in ovariectomized mice exposed to 16.7 mg/kg-day for three days (Tullner, 1961). This technique is a relatively sensitive assay for estrogenicity; the ability of a chemical to replace natural estrogens in a primed, estrogen-deficient animal is not, strictly speaking, an adverse effect. Increased vaginal cornification was observed in female rats exposed to 50-200 mg/kg-day for several days beginning on postpartum day 21 (Gray *et al*., 1989). An enlarged uterus was observed in rats exposed to 150 mg/kg-day for six weeks (Harris *et al*., 1974) and in pigs exposed to 1,000 mg/kg-day for 24 weeks (Tegeris *et al*., 1966). Mammary gland hyperplasia was also observed in the pigs. Repeated doses of 50-400 mg/kg-day MXC produced atrophic changes in ovaries of mice and rats similar to those produced by estrogens (Bal, 1984; Gray *et al*., 1988, 1989; Martinez and Swartz, 1991).

MXC effects on estrous cyclicity can adversely affect reproductive function and decrease fertility. Female rats exposed to 100 mg/kg-day MXC beginning on postpartum day 21 showed a 40 percent decrease in fertility and live pups/litter when mated with untreated males, and an 80 percent decrease when mated with similarly-treated males (Gray *et al*., 1989). A higher MXC dose (200 mg/kg-day) produced infertility in 100 percent of the animals; lack of implantation sites indicated that the effect occurred prior to implantation. Infertility was also observed in female rats following intermediate-duration exposure to 100 mg/kg-day (Bal, 1984). Decreased fertility was observed in rats exposed to 50 mg/kg-day for three generations, but not at 10 mg/kg-day (Haskell Laboratories, 1966; probably same study as du Pont, 1966). In female rats, a decreased mating frequency and a decreased fertility in those that mated were noted following exposure to 60-150 mg/kg-day (Harris *et al*., 1974). Increased resorptions have been consistently reported in rats following acute and intermediate-duration exposures to 35.5-200 mg/kg-day MXC

(Harris *et al*., 1974; Culik and Kaplan, 1976; Kincaid Enterprises, 1986; Cummings and Gray, 1989; Gray *et al*., 1989; Cummings and Perreault, 1990). Acceleration of embryo transport into the uterus appears to be one mechanism responsible for increases in preimplantation loss (Cummings and Perreault, 1990).

Exposure of female mice to MXC during pregnancy affects reproductive parameters not only in female offspring exposed prenatally, but also in those of a subsequent litter not directly exposed to MXC (Swartz and Corkern, 1992). Pregnant young adult CD-1 mice (n=8 per group) were administered either 2.5, 5.0, 7.5 mg technical grade MXC or 0.025 mg estradiol (positive control) via oral gavage from days 6-15 of pregnancy. These doses of MXC were equivalent to approximately 100, 200 or 300 mg/kg, respectively. Control mice received sesame oil. All were first time breeders. Female offspring (F_{1a}) were cross-fostered within 48 hours of birth (this was done to determine the relative importance of prenatal and/or postnatal exposure of MXC on observed toxic reproductive effects). Female offspring were weaned at PND 21 and were killed at 8-10 weeks. Ovaries were obtained from animals in estrus on the day they were sacrificed for histological evaluation. All statistical analyses employed ovarian weights expressed in relation to the total body weight. The ovaries of the groups were compared for statistical analysis. Following weaning of the first litter, the dams were allowed to mate again and deliver a second litter (F_{1b}). The second litter was evaluated in a manner similar to that for the F_{1a} litter. MXC treatment had no effect on maternal weight gain during pregnancy. No maternal toxicity was noted at any of the doses tested. Dams exposed at the 2.5 and 5.0 mg MXC dosages carried their offspring to term, but MXC was embryotoxic at the 7.5 dosage level. Pregnant mice exposed to either estradiol or to 5.0 mg MXC experienced a significant delay in the time of delivery; no differences in the number or weight of the offspring were observed between exposure groups. No significant differences were found in ovarian weights between treated groups and controls of the F_{1a} generation. No significant differences in the time of appearance of vaginal openings were found among any of the F_{1a} generation. A small number of polyovular follicles were found in six of nine 5.0 MXC-exposed F_{1a} offspring – this is considered an uncommon finding. A higher incidence of atresia in large follicles was found in F_{1a} mice exposed to 2.5 and 5.0 mg MXC (since the large follicles comprise the pool from which ovulated oocytes will arise, the increase in atresia in this group may affect the immediate fertility of the animal). Female F_{1b} offspring of mothers exposed to 2.5 and 5.0 MXC (100 and 200 mg/kg, respectively) showed a significant advancement ($p<0.05$) in the day of vaginal opening, 23.9 ± 0.3 days and 23.3 ± 0.3 days for 2.5 and 5.0 MXC, respectively, vs. 25.0 ± 0.2 days for control animals.

Exposure of female mice to MXC early in life produced significant alterations in ovarian morphology and fertility at sexual maturity (Swartz and Eroschenko, 1998). Beginning at one day of age, female CD-1 mice were exposed intraperitoneally (ip) for fourteen consecutive days to either sesame oil, 10 µg estradiol, or 0.1, 0.5, or 1.0 mg technical MXC. Due to the increase in the animals' body weight over time, these doses corresponded to a range during the experiment of 14 to 71, 68 to 357, or 135 to 714 mg MXC/kg, respectively. The estradiol-exposed group served as a positive control. The chemicals were administered *ip* because the one-day old mice were too small to be gavaged. The dose levels for this study were selected based on previous observations

that these doses produced a range of dose-dependent alterations in the reproductive systems of immature and mature female mice without mortality or altering body weights. Female offspring were weaned at 21 days, and at three months of age were placed with proven breeder males. Females were sacrificed after 18 days of gestation. Uterine horns were examined for fetuses and resorption sites. Animals that failed to mate within the two-week period also had their ovaries removed for evaluation. At three months of age, mating ability was not inhibited in any of the MXC-treated groups (one female exposed to 1.0 mg MXC failed to mate within the two-week time period). In the group exposed to 0.1 mg MXC, 85.7 percent of the females became pregnant, whereas in the 0.5 and 1.0 mg groups, 75 and 25 percent, respectively, became pregnant, a significant decrease only at 1.0 mg MXC. The mean number of live fetuses/litter was reduced in the 0.5 and 1.0 mg MXC-treated groups. Corpora lutea were significantly reduced in ovaries from only the 1.0 mg MXC group. No effects of treatment were seen at 0.1 mg MXC.

Uzumcu *et al.* (2006) showed that early postnatal MXC exposure inhibits folliculogenesis and stimulates anti-Mullerian hormone (AMH) production in the rat ovary. Neonatal female Sprague-Dawley rats were injected daily (s.c.) with 1, 10, 50, 100 or 500 mg/kg-day MXC from PND 3 to PND 10. Control rats were injected with vehicle, 25 µl dimethyl sulfoxide:sesame oil. In the rat neonate, PND 3-10 represents the period of primordial-to-primary follicle transition (early folliculogenesis). For the studies on ovarian weight, histology, immunohistochemistry, follicle number, and whole ovary AMH western blot analysis, experiments were repeated two to five times, using at least two to three animals for each treatment in each experimental repeat. Therefore, five to eleven animals were used for each treatment group.

None of the MXC doses caused systemic toxicological effects as indicated by general appearance, and no significant change in body weight occurred. MXC treatment caused a reduction in the size of the ovary and inhibited folliculogenesis. Daily MXC treatment from PND 3 to 10 caused a significant decrease in ovarian weight for 50, 100 and 500 mg/kg-day groups, by 35, 75 and 80 percent, respectively. A significant increase in uterine weight was observed at 100 and 500 mg/kg-day. There was a dose-dependent reduction in the number of antral follicles in the ovaries of 50, 100 and 500 mg/kg-day MXC animals; the 50 mg/kg-day dose eliminated most of the large antral follicles, while ovaries of 100 and 500 mg/kg-day groups had few or no antral follicles. The ovary size, histology and follicle composition of 1 and 10 mg/kg-day groups were similar to those of controls.

In addition, MXC treatment increased AMH production in the ovary (AMH suppresses initial follicle recruitment in the ovary, *i.e.*, inhibits folliculogenesis). AMH protein production from ovaries of 1 and 10 mg/kg MXC-treated females was not significantly increased from control ovaries, whereas in ovaries from 50, 100 and 500 MXC-treated females, the increases were statistically significant ($p<0.05$). Estrogen and estrogenic compounds, such as MXC and its metabolites, are known to regulate ovarian AMH gene expression. The LOAEL for this study is identified as 50 mg/kg-day (significant decrease in ovarian weight, reduction of antral follicles in the ovary, inhibition of follicular development). The NOAEL is 10 mg/kg-day (minimal ovarian changes, not significantly different from controls).

Changes in sex hormone levels are also observed after MXC treatments. Decreased serum progesterone levels were observed in female rats at 50-100 mg/kg-day, but not at 25 mg/kg-day (Cummings and Gray, 1989; Cummings and Laskey, 1993). Pituitary levels of prolactin were decreased in intact female rats but increased in ovariectomized rats exposed to 400 mg/kg-day (Gray *et al*., 1988). Martinez and Swartz (1992) speculated that MXC causes a feedback inhibition of pituitary hormone secretions, resulting in a lack of stimulation of ovarian cells to produce their usual hormones, which sustain the function of the uterus and other reproductive tissues.

Masutomi *et al*. (2003) fed female Sprague-Dawley rats (n= 5-6 dams/group) a pelleted diet containing either MXC (0, 24, 240, or 1,200 ppm), genistein or diisononyl phthalate from gestational day (GD) 15 to postnatal day (PND) 10 (the critical period for the pups' brain sexual differentiation). Only the results in female offspring are discussed here (also see the reproductive effects in males section below). Maternal body weights were not reported in this study. Estimating maternal body weight at 250 g and feed consumption at 5 g/100 g of body weight (both taken from the Charles River Labs website), MXC doses would be approximately 1.2, 12, and 60 mg/kg-day, respectively**.**

All dams delivered live pups. Litter size was not affected by any dose in the MXC studies compared to corresponding controls. Neonatal body weights of offspring of the 1,200 ppm MXC group, measured on PND 2, showed a non-significant tendency for decrease. From PND 2 to PND 10, reduction of body weight of offspring (of both sexes) was obvious in 1,200 ppm MXC-exposed animals. Recovery of body weights was noted after cessation of exposure. At PND 21, the offspring were weaned and grouped as follows: five males and five females (one female and one male per litter) per dose group for prepubertal necropsy; and eight males and eight females (at least one male and one female per litter) per group for adult examination. Examination of female offspring included measurement of anogenital distances (AGD), prepubertal organ weights, onset of puberty, estrous cyclicity, and organ weights and histopathology of endocrine organs at week 11 (the adult stage), as well as determination of the volume of the sexually dimorphic nucleus of the brain preoptic area (SDN-POA).

Effects in female offspring included early onset of puberty, histopathological alterations in the reproductive tract and anterior pituitary, and irregular estrous cyclicity. These changes are identical to those previously reported for MXC by other investigators (Chapin *et al*., 1997; Newbold, 1999). Ovaries of all 1,200 ppm MXC female offspring, including those exhibiting regular estrous cyclicity, showed an increase of follicles and decrease of corpora lutea. In the uterus, hypertrophy of both luminal and glandular epithelia was evident, and squamous metaplasia developed in one case. Hyperplasia of vaginal epithelia was evident and some cases showed mucinous degeneration associated with scattered single-cell keratinization.

In addition to changes in the reproductive tract, a marginal, non-significant increase in the incidence and severity of lobular hyperplasia of the mammary gland was observed in the highest dose group. Pituitary weights of female offspring with irregular estrous cycles were increased (138 percent of control value) with diffuse hyperplasia evident in the anterior lobe. The changes were profound in animals showing irregular estrous cycling. Female offspring fed either 24 or 240 ppm (1.2 or 12 mg/kg-day) MXC showed

no histopathological lesions in any organs examined. Weights of ovaries were not significantly decreased. No differences were found in SDN-POA values between controls and each treatment group for either sex when measured at postnatal week 11. The authors suggest that measurement of the SDN-POA volume may not be sensitive enough to detect weak hormonal influence on brain sexual differentiation. The lowest dose at which toxic and/or reproductive effects were seen in dams and offspring was 1,200 ppm (~ 60 mg/kg-day) MXC. Treatment-related effects at this dose included an increase in ovarian follicles, a decrease in corpora lutea, hypertrophy of uterine epithelia, hyperplasia of vaginal epithelia, and increases in pituitary weight and hyperplasia. The NOAEL for this study is approximately 12 mg MXC/kg-day.

There is considerable evidence from both *in vivo* and *in vitro* studies that hormones and endocrine-disrupting chemicals can exert stimulatory effects at low doses and inhibitory effects at high doses (*i.e.,* that the direction of the effect of exogenous estrogenic chemicals on hormone receptors is dependent on dose). This may be due to competition among endogenous and exogenous estrogenic chemicals with different affinities and efficacies on the hormone receptors; the net effects (additive, competitive, or antagonistic) would depend on relative receptor occupancy. In other cases, a mixed effect might occur because of action at two or more receptors, such as estrogen and androgen receptors.

Alworth *et al*. (2002) evaluated the effects on the response of the adult uterus to estradiol of high and low-dose MXC exposure during fetal life. The estrogenic compound diethylstilbestrol (DES) served as a positive control. Pregnant female CD-1 mice were administered orally either 0.1 or 100 µg/kg-day of DES, or either 10 or 10,000 µg/kg-day MXC on gestation days 12-18. (In mice, the gonads begin differentiating on GD 12, and the accessory reproductive organs begin differentiating on GD 15). At 7-8 months of age, female offspring were ovariectomized and implanted for seven days with a Silastic capsule containing estradiol, to decrease variability in estradiol levels and estrogen-responsive tissues. In experiment 2, using 0.5 µg estradiol, female offspring exposed prenatally to the 10,000 µg/kg dose of MXC had significantly lighter uteri than females exposed to the 10 µg/kg dose ($p < 0.05$), although neither group differed significantly from controls. Females exposed to the 10 µg/kg MXC dose tended to have heavier uterine weight relative to controls, but this was not significant. Statistically significant differences were seen between the low and high DES groups in the uterine response to estradiol, with the uterine weights at the low DES dose significantly higher and the high DES dose significantly lower than controls. In experiment 3, no differences in uterine weight for the two doses of MXC were seen in rats implanted with 0.25 µg or 0.5 µg estradiol, but the same general effect was seen at 1.0 µg estradiol as in experiment 2. That is, there was a significant difference in uterine weight between the two MXC doses, but neither was significantly different from controls.

Body weights did not differ from controls for females exposed prenatally to MXC. Female offspring exposed to the low dose of MXC tended to show an increase in liver weight ($p=0.06$) relative to controls in experiment 2, but had a non-significant decrease in experiment 3. Both the low and high prenatal DES doses significantly increased adult liver weight relative to controls. The authors suggest that enhanced responsiveness of the uterus to estradiol due to developmental exposure to a low dose of an estrogenic chemical

may be caused by a permanent up-regulation of uterine estrogen receptors, whereas decreased responsiveness of the uterus after developmental exposure to much higher doses may be caused by a permanent down-regulation of estrogen receptors.

The onset of puberty is regulated differently in primates than in rodents (Mann and Plant, 2002; Terasawa and Fernandez, 2001). Rhesus monkeys are a standard model for human health research. Like humans, female monkeys have a long and complex period of maturation during adolescence. Golub *et al.* (2003) studied the effects of exogenous estrogenic agents on pubertal growth and reproductive system maturation in female rhesus monkeys. Prepubertal female Rhesus monkeys (n=8 per treatment group) were dosed daily for one year with either 25 or 50 mg/kg MXC, 0.5 mg/kg diethylstilbestrol (DES) or vehicle control. Only the results for MXC are reported here. MXC was mixed with fruit-flavored baby food and administered orally. Doses were selected based on earlier studies (Chapin *et al.*, 1997). The MXC25 exposure group lagged behind controls in weight gain at the peak of the growth spurt (study month 4-10) and the MXC50 group lagged behind controls towards the end of the growth spurt. Height growth was lessened in the MXC25 group; trunk growth appeared more affected by MXC than long bone growth. MXC-induced growth retardation has been reported in rats (Chapin *et al*., 1997, Gray *et al*., 1999).

The nipple volume (the major external secondary sex characteristic to develop in rhesus monkeys at puberty) was significantly smaller in the MXC50 group at the end of treatment compared to controls. Another puberty-related morphological change, sex skin swelling and reddening, appears after puberty in rhesus monkeys. Nonperineal sex skin was more frequently detected in the MXC25 group than in controls; the MXC50 group was less affected. Sex skin on the sides of the trunk was detected with MXC treatment, but not with controls. The MXC50 treatment group showed a delay in onset of menarche (5.2 ± 0.6 months vs. 3.4 ± 0.7 months in controls). In addition, MXC-treated groups did not show the decrease in cycling typically seen during the summer months in rhesus monkeys. Treatment-related effects were detected eight months after cessation of dosing. Ovulatory cycling was examined at the end of the recovery period through analysis of daily urine samples for 60 consecutive days. There were fewer monkeys with normal cycles in the treated groups than in the control group. MXC-treated monkeys tended to exhibit short follicular phases (estrogen peak <11 days post menses). The results from the *in vitro* activation assay indicated that serum from MXC-treated animals had increased ability (about 50 percent higher) to activate gene expression through the ERa receptor compared to controls. A potentially important finding of the study was that the lower dose, MXC25, had an apparently greater estrogenic effect on some endpoints (height growth, early appearance of perineal sex skin, incidence of nonperineal sex skin during treatment, and ovarian structural changes) than the higher dose (50 mg/kg-day).

MXC induces significant oxidative stress DNA damage in the mouse ovarian surface epithelium (OSE) *in vitro* (Symonds *et al.*, 2008). The OSE is of particular interest as a major source of ovarian cancer. Female FVB mice were used in all experiments. Mouse OSE cells were treated with either MXC, 17β-estradiol (E_2) $\pm$ the anti-oxidant vitamin E, progesterone, or hydrogen peroxide (H_2O_2) (positive control). The cells were then subjected to an immunofluorescent assay that detects oxidative damage to DNA. (The altered nucleoside, 8-OH-dG, is a major product of oxidative damage to DNA and

represents a marker for such damage). Short-term incubation with MXC did not significantly affect levels of 8-OH-dG in the OSE. However, longer-term exposure to MXC produced a 6-fold greater signal than in untreated controls at 24 h exposure, and a two-fold elevation at 72 h. This damage was prevented by the anti-oxidant vitamin E. Prior studies on the effect of MXC on the OSE demonstrated proliferative effects, diminished apoptosis, genomic expression of cell cycle regulators, Bcl-2, Bax, and cytochrome P450 (CYP450) enzymes (Symonds *et al.,* 2005, 2006). Other authors (Gupta *et al.,* 2006) have similarly reported that MXC induces oxidative stress damage to the ovary.

Reproductive Effects in Male Animals

Oral exposure to MXC can produce gross and histopathological changes in the male reproductive system. Reported effects of methoxychlor on male reproduction include delayed sexual maturity, decrease in testis weight, atrophy of the epididymis, prostate and seminal vesicles, impaired steroidogenesis, decreased epididymal sperm count, and viability at doses between 20-500 mg/kg-day (Okazaki *et al.,* 2001). Estrogenic effects on testes during critical developmental periods have been suggested as a potential cause of reproductive impairments (Toppari *et al*., 1996) or cancer (McLachlan *et al*., 1998).

Decreased testes weight was observed in male rats and mice exposed to 50-1,400 mg/kg-day of MXC (Wenda-Rozewicka, 1983; Bal, 1984; Gray *et al*., 1989; Chapin *et al*., 1997). Several reproductive organs (testes, epididymis, seminal vesicles, and prostate) exhibited significantly lower weights in male rats exposed through their dams to 50 or 150 mg/kg-day from gestational day 14 to postnatal day 7, then gavaged directly with the same dose through day 46. Decreased prostate weight was observed in male rats exposed to 154 mg/kg-day for 90 days (Shain *et al*., 1977), and a decreased caudal epididymal sperm count was observed in rats exposed to 50-100 mg/kg-day starting at postnatal day 21 (Gray *et al*., 1989).

Judy *et al*. (1999) gave pregnant CF-1 mice daily oral doses of 0, 20 or 2,000 µg/kg-day of MXC in 30 µL of corn oil from GD 11 to GD 17. When male offspring reached adulthood (9.5 months old), they were killed and the prostate, seminal vesicles, preputial glands, liver, and adrenals were removed and weighed. One male was evaluated from each of 9, 6, or 5 litters for the respective doses. Fetal exposure to MXC in this study resulted in a significant *increase* in adult prostate weight (both doses) and seminal vesicle weight (2,000 µg/kg-day only). Liver weights were slightly decreased by both doses. Neither dose of MXC altered body weight. Body weight accounted for a significant portion of the variance for the seminal vesicles ($p<0.05$) and liver ($p<0.001$), and was marginally related to prostate weight ($p<0.06$) and testis weight ($p=0.07$). Body weight was unrelated to adrenal weight ($p>0.1$) and preputial gland weight ($p>0.1$). Table 5 shows the mean weights for organs which showed significant changes. The LOAEL for this study was 20 µg/kg-day (lowest-dose tested).

Table 5. Mean Organ Weights (± SEM) in 9.5-Month-Old CF-1 Mice Exposed Prenatally to Methoxychlor (adapted from Judy *et al*., 1999)

Dose Group (μg/kg)	No. of Animals	Body weight (g)	Prostate (mg)	Seminal Vesicles (mg)	Liver (g)
Control	9	39.2 ± 1.1	40.0 ± 3.0	66.3 ± 3.7	2.26±0.03
20	6	38.6 ± 1.3	64.5 ± 3.7**	77.3 ± 4.5	2.15±0.04*
2000	5	37.4 ± 1.5	60.3 ± 4.1**	79.5 ± 5.0*	2.12±0.04*

Means presented for the organ weights are adjusted for the effect of body weight by analysis of covariance. * = $p < 0.05$; ** $p < 0.001$.

It should be noted that the effects on prostate in this study were in the opposite direction of those noted in the earlier studies, at much higher doses. This appears unlikely to be an artifact, but rather is due to the mechanism of action of this and other endocrine-disruptive compounds, exhibiting a U-shaped dose-response curve (Judy *et al*., 1999, Alworth *et al.*, 2002, Welshons *et al.*, 2003).

Chapin *et al*. (1997) found that exposure to MXC adversely affects reproductive development or function in male animals. Preputial separation was significantly delayed in male rats exposed to 50 or 150 mg/kg-day through their dams from gestational day 14 through postnatal day 7, then directly gavaged from postnatal day 7 through day 42. This suggests that sexual maturity may be delayed.

Doses of 100 or 200 mg/kg-day of MXC beginning on postpartum day 21 (Gray *et al*., 1989) similarly delayed preputial separation. Fertility was decreased by 80 percent when males rats exposed to 100 mg/kg-day were mated with similarly treated females, compared to only a 50 percent decrease when untreated males were mated with treated females (Gray *et al*., 1989). Decreased fertility was also reported in male mice treated with 60 mg/kg-day of MXC (Wenda-Rozewicka, 1983) and in male rats at 150 mg/kg-day in the studies of Chapin *et al.* (1997). In addition, mating frequency and fertility in male rats that mated were significantly reduced after exposure to MXC in utero, during lactation, and/or postweaning at 60 mg/kg-day (Harris *et al*., 1974).

Perinatal and juvenile exposure of rats to MXC during development reduces the overall functional spermatogenic capability of the testis in adult animals (Staub *et al*., 2002). Rat dams were gavaged with MXC at 0, 5, 50 or 150 mg/kg-day for the week before and after they gave birth. Resulting male pups (14-16 per group) were then dosed directly from PND 7 to 42.

Across dosage groups, testicular weight was significantly reduced in a dose-dependent fashion. The number of spermatogonia and spermatids per testis was significantly reduced by treatment, with the two highest dose groups having fewer surviving spermatids ($p < 0.01$) than the control or low dose groups. Similarly, the three MXC-treated groups had a reduced number of spermatogonia per testis and per gram of testicular parenchyma ($p < 0.01$) than the controls. The three MXC-treated groups had fewer spermatogonia per Sertoli cell than did the control group. When the types of spermatogonia were identified, it appeared that MXC particularly affected early

spermatogonia. In this study, one measure of efficiency of spermatogenesis (daily sperm production per gram of testicular parenchyma) was not affected. Surviving spermatogonia were not adversely affected, as they had a significantly higher survival rate after treatment (with the exception of the 150 mg MXC/kg-day dose group, which was not different from the control). Staub *et al*. (2002) concluded that the fact that surviving spermatogonial progeny were able to compensate (by reducing the normal amount of germ cell degeneration), to recover the spermatogenic potential per gram, indicates that treatment effect evaluated later in spermatogenesis may not indicate the damage done during spermatogonial development. No effect on the number of spermatids per gram does not indicate that there was no effect on spermatogonia. Statistically-significant treatment-related effects (fewer spermatogonia per testis and per gram of testicular parenchyma ($p < 0.01$), reduction in testes weight) were observed at all doses tested in this study, hence the LOAEL for this study is 5 mg/kg-day MXC.

Masutomi *et al*. (2003) fed female Sprague-Dawley rats (n=5-6 dams/group) either MXC (0, 24, 240, or 1,200 ppm), genistein or diisononyl phthalate from gestational day (GD) 15 to postnatal day (PND) 10 (the critical period for brain sexual differentiation of offspring). Only the results for MXC in male offspring are discussed here (see reproductive effects in females above for effects on female offspring). Maternal body weights were not reported in this study. Estimating maternal body weight at 250 g, and a feed consumption rate of 5 g/100 g body weight (values from Charles River Labs growth website), MXC doses would be approximately 1.2, 12, and 60 mg/kg-day, respectively.

At PND 21, the offspring were weaned. Five males (one per litter) were used for prepubertal necropsy and eight (at least one per litter) examined as adults. Offspring were examined for anogenital distances (AGD), prepubertal organ weights, onset of puberty, and organ weights and histopathology of endocrine organs at week 11 (adult stage). The volumes of the sexually dimorphic nucleus of the brain preoptic area (SDN-POA) were also measured. All dams delivered live pups, and litter size was not affected by any MXC dose compared to corresponding controls.

Neonatal body weights of offspring of the 1,200 ppm MXC dose group, measured on PND 2, showed a non-significant tendency for decrease. From PND 2 to PND 10, reduction of body weight of offspring (of both sexes) was obvious in the 1,200 ppm MXC-exposed animals. Recovery was noted after cessation of exposure. In addition, the 1,200 ppm MXC group showed a non-significant tendency for body weight reduction at prepubertal necropsy. Organ weight changes were also noted in these cases, testes weights being most markedly affected in the 1,200 ppm MXC group. Also in the 1,200 ppm MXC group, male offspring showed about a 2-day delay in onset of preputial separation. Slight reductions in testicular weights were observed for animals in the 24 or 1,200 ppm MXC-exposed groups on PND 21. No lesions were found in either adult testes or in other reproductive organs and the pituitary. No differences were found in SDN-POA values between controls and each treatment group (measured at postnatal week 11). The authors suggest that the volume measurement of the SDN-POA may not be sensitive enough to detect weak hormonal influence on brain sexual differentiation (sexual development becomes especially sensitive to estrogenic stimuli during the juvenile period after PND 10). The lowest dose at which toxic and/or reproductive effects were seen in dams and offspring was 1,200 ppm (~ 60 mg/kg-day) MXC.

Treatment-related effects at this dose level included decreased maternal body weight gain, as well as reproductive changes (reduction in testes weight and delay in onset of preputial separation) in male offspring. The NOAEL for this study is approximately 240 ppm MXC (12 mg/kg-day).

Prenatal exposure to very low doses of MXC in utero has been shown to markedly alter social-sexual behavior in male mice (vom Saal *et al.,* 1995). Pregnant CF1 mice were orally administered 0, 1, 100, or 5,000 µg MXC/day in 30 µl tocopherol-stripped corn oil (n = 6-10 females/group) on GD11 through GD17 via an electronic micropipetter. (This procedure does not result in the severe stress associated with gavage. It has been reported that severe maternal stress significantly alters fetal steroid hormone levels and, consequently, the course of fetal development [Vom Saal *et al*., 1990]). Tocopherol-stripped corn oil was used as the vehicle because co-administration of the anti-oxidant Vitamin E in MXC-treated rats has been shown to prevent significant oxidative stress in reproductive tissues (Latchoumycandane *et al.,* 2002). Maternal body weights in the current study ranged from 45 to 65 g from days 11 to 17. Thus, the corresponding calculated mg/kg-day doses would be about 0.02, 2, and 100 mg MXC/kg-day (the two lower doses are as reported in Judy *et al.*, 1999, in a study using the same administration technique, possibly involving different offspring from the same treated dams).

Open-field urine marking was studied in two male mice per litter at 60 days of age, n = 24 control mice and 10 for each MXC dose group. Urine-marking plays a major part in determining reproductive success in male mice. A stable mouse population requires that a single male within a small family of mice be the dominant territory-marking male. Changes in social-sexual behaviors can result in marked disturbances in social structure, and may be associated with lower reproduction and a decrease in population size. Increasing urine marking was observed at the three doses (0.02, 2, and 100 mg/kg) tested, compared to control. Urine-marking behavior in these male offspring was significantly increased at the lowest MXC dose, 0.02 mg/kg-day, which represents the LOAEL for this study. The authors concluded that behavioral changes appear to represent the most sensitive endpoint for endocrine-disrupting chemicals.

MXC also produces changes in hormone levels in male animals. Exposure to 25-50 mg/kg-day MXC increased levels of prolactin, follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH) in the pituitary of male rats (Goldman *et al*., 1986; Gray *et al*., 1989). Serum levels of TSH, testosterone, and progesterone were decreased in rats dosed with 100 mg/kg-day of MXC (Cummings and Gray, 1989; Gray *et al*., 1989). Similarly, reduced levels of testosterone were reported in the interstitial fluid and epididymis of male rats treated with 100 mg/kg-day of MXC (Gray *et al*., 1989).

Short-term exposure of young adult male rats to MXC has been shown to inhibit Leydig cell testosterone biosynthesis (Murono *et al*., 2006). Forty-eight day old male Sprague-Dawley rats were administered 0, 5, 40 or 200 mg/kg MXC by gavage for 7 consecutive days. Reported results are the mean $\pm$ standard error of at least 13 animals per treatment group. Although testicular weights among the four treatment groups were not significantly different, fluid-retained and fluid-expressed seminal vesicle weights declined to 44 and 60 percent of control, respectively, in the 200 mg/kg MXC-treated animals. Both fluid-retained and fluid-expressed weights were determined to assess

whether any potential changes in seminal vesicle weights represented more than the amount of seminal fluid produced or retained by the glands. In the 200 mg/kg MXC-treated group, serum testosterone levels declined to 41 percent of control (1.82 $\pm$ 0.27 ng/mL vs. 4.40 $\pm$ 0.52 ng/mL) and serum dehydroepiandrosterone (DHEA) levels declined to 45 percent of control. There was a tendency for both DHEA and testosterone levels to be lower than control in the 40 mg/kg MXC treatment group. The decline in testosterone appears to be related to a dose-dependent decline in Leydig cell P450 cholesterol side-chain cleavage (P450scc) activity at the 40 and 200 mg/kg doses. Treatment with 200 mg/kg MXC lowered Leydig cell testosterone production to ~ 49 percent of control; treatment with 5 or 40 mg/kg MXC had no effect on this parameter. No differences were seen in serum corticosterone, leutinizing hormone or FSH levels between treated and control groups, which suggests that MXC and/or its metabolites were acting directly on the testis. These *in vivo* studies support previous *in vitro* studies on isolated Leydig cells from adult rats (Alingbemi *et al*., 2000; Murano and Derk, 2004).

Vaithinathan *et al.* (2008) studied the effects of a single low dose of MXC on testicular steroidogenesis in rats. Adult male Wistar rats (4/group) were given an oral dose of MXC at the reported LOAEL for rats of 50 mg/kg (WHO, 1996) and were killed 0, 3, 6, 12, 24 or 72 hours later. (MXC is reported to be excreted to a large extent within 24 hours of oral exposure.) The activities of 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β – hydroxysteroid dehydrogenase (17β-HSD), levels of hydrogen peroxide, the expression of steroidogenic acute regulatory (StAR) protein, and androgen binding protein (ABP) were measured in testes. H_2O_2 was measured because it is known to exhibit antisteroidogenic effects (Stocco *et al.*, 1993). StAR protein was monitored to evaluate the effect of MXC on cholesterol utilization by Leydig cells.

MXC administration resulted in a sequential reduction in expression of StAR protein and activities of 3β-HSD and 17β-HSD, with a concomitant increase in testicular hydrogen peroxide. These changes were significant between 6 and 12 hours following treatment. By 72 hours, activity of 3β-HSD and 17β-HSD, as well as levels of H_2O_2, returned to near basal levels. Treatment with MXC resulted in a significant decrease in ABP at 6-12 hours following treatment, recovering to near basal levels by 3 days post-treatment. The authors conclude that transient but significant effects of MXC on testicular steroidogenesis could perturb spermatogenesis.

A single oral dose of MXC (50 mg/kg body weight) induced alterations in the levels of stress proteins, heat shock proteins (HSP), clusterin and oxidative stress-related parameters in the testis of adult male rats (Vaithinathan *et al.*, 2009). Adult male Wistar rats (4/group) were gavaged with 0, 5, 25, 50, 100, or 200 mg/kg MXC, and the lowest dose that caused significant changes in stress parameters was selected. Animals were killed at 0, 3, 6, 12, 24 and 72 hours after dosing. Testes were collected and lysates prepared for analysis. The activities of catalase and superoxide dismutase (SOD) in the testis of rats exposed to 50 mg/kg MXC were decreased in a time-dependent manner compared with the control group. Peak reductions for both were observed at 6 hours. By 72 hours, the activities of catalase and SOD returned to near basal levels. Lipid peroxidation in MXC-treated rats increased 6-12 hours after exposure and returned to near basal levels by 72 hours. Immunoblot analysis of HSP revealed the expression of HSP72, an inducible form of HSP, at 3 to 24 hours following MXC treatment. (Under

physiological conditions, constitutively expressed HSP72 acts as a molecular chaperone that assists in proper folding, assembly, and intercellular trafficking of newly synthesized proteins). Similarly, secretory clusterin was also elevated 3-12 hours after treatment. Clusterin, a heterodimeric glycoprotein, is produced by Sertoli cells and is widely distributed in many tissues. Clusterin is differentially regulated in many pathological states that are characterized by significant oxidative injury.

The endocrine effects of MXC can be influenced by the presence of additional endocrine-active compounds. In one study in rats, You *et al.* (2002) found that combined exposure to the estrogenic isoflavone genistein (GE) (found in soybeans and other legumes) and MXC promoted the development of alveolar-lobular structure of the mammary gland. This effect was not observed with either compound alone, and is not normally seen in male mammary gland. Pregnant Sprague-Dawley rats (n=8 dams/group) were fed a soy- and alfalfa-free diet containing different combinations of GE (300 and 800 ppm) and MXC (800 ppm) from GD1, continuing through pregnancy and lactation, until PND 21, when the pups were weaned. Offspring continued to be fed with the respective maternal diets throughout the study. The estimated doses of GE and MXC are shown in Table 6, based on feed consumption over 3-4 day periods at various developmental stages. While exposure levels were relatively consistent for the pregnant dams, the doses were much higher for the offspring at the prepubertal stage than at adult stages.

Table 6. Estimated Doses (mg/kg-day) of Dams and Male and Female Offspring Exposed to Genistein and Methoxychlor (adapted from *You et al.*, 2002)

	Dam				Male offspring			Female offspring		
Dams (GD)/ Offspring (PND)	1-3	3-7	7-11	20-23	28-31	55-58	97-100	28-31	55-58	97-100
Methoxychlor (800 ppm)	43.8 ± 9.6	61.0 ± 7.4	63.5 ± 8.1	54.4 ± 8.4	126 ± 8.5	70.6 ± 8.6	46.3 ± 4.7	116 ± 16	68.4 ± 4.6	56.3 ± 8.4
Genistein (300 ppm)	26.9 ± 1.6	30.2 ± 1.8	26.5 ± 1.3	20.7 ± 4.5	44.5 ± 2.8	26.2 ± 1.1	15.7 ± 1.0	39.6 ± 6.2	22.5 ± 2.5	16.8 ± 2.5
Genistein (800 ppm)	64.1 ± 15.3	70.4 ± 7.5	68.8 ± 4.7	57.0 ± 14.2	125 ± 10	70.6 ± 7.9	43.1 ± 5.1	116 ± 7	71.1 ± 5.6	49.2 ± 6.2

Data are mean ± SD (n = 8 litters for all groups); litter means were used for the offspring data.
GD = gestational day; PND = postnatal day.

On PND 22, one pup of each sex per litter was killed and necropsied. Starting on PND 25, until completion, all the remaining female offspring (average, 5 per litter) were examined daily for vaginal opening (VO). Starting on PND 35, all the remaining males (average 4.4 per litter) were examined daily for preputial separation (PPS). Estrous cyclicity was determined in two adult female offspring randomly selected from each litter (n= 6-8 litters per dietary group). Motor activity was measured in one rat per sex of each litter on PND 64-65 (n= 6-8 litters per treatment group). On PND 110, adult male and female offspring (mostly 3 males and one female per litter) were killed and necropsied.

Values were expressed as means ± standard deviations when appropriate. When littermates were included in a measurement, the data presented are group means, and their standard deviation was derived from litter averages.

No statistically significant treatment effect was observed in male or female offspring for anogenital distance (AGD). Both MXC and GE reduced feed consumption of the pregnant dams during gestation ($p<0.01$ for both treatments); 800 ppm MXC reduced feed consumption 20-34 percent, while 800 ppm GE reduced it 9-21 percent from the beginning of gestation through lactation; no effect on feed consumption occurred with 300 ppm GE. When administered together, the effects of the two compounds in reducing feed intake appeared to be additive. The feed intake of both sexes of the offspring was affected by both MXC and GE ($p<0.01$ for both sexes with both compounds).

Body weights of the dams were significantly reduced in the MXC-fed group ($p<0.01$) but not in the GE-fed groups. Both MXC and GE significantly decreased the body weight of the female newborns ($p<0.01$ for both factors), with the effect of both the 300 and 800 ppm GE being significant ($p<0.01$); the weights of male newborns were not affected by any treatment. The effect of MXC and GE on the rate of body growth was pronounced in both sexes of offspring, although the effect was more severe on females than males. At PND 21, male organ weights were not significantly affected by the treatment. However, mean uterus weights were more than doubled in the MXC-treated rats ($p<0.01$); the GE treatment did not result in a significant effect in this regard. PPS was delayed in MXC-treated offspring ($p<0.01$). GE alone was not effective in delaying PPS, but enhanced the potency of MXC in delaying male pubertal development when co-administered with MXC. The effect of both compounds on accelerating VO was significant ($p<0.01$ for both factors); on the first day of the examination, some of the MXC-treated female offspring had already become VO-positive. In contrast, no rat in non-MXC treated groups displayed vaginal patency at PND 25. MXC-treatment increased the proportion of time that the females were in estrus ($p<0.01$) and decreased their proportion of time in diestrus ($p<0.01$); no significant effect was seen in this regard with GE.

GE and MXC both caused changes in the developmental pattern of the prepubertal mammary glands. The total glandular area and the number of branch points, lateral buds, and terminal end buds in the male rats were found to be significantly greater in the groups exposed to MXC than those exposed to GE only. These effects were not observed in female rats. In the male rats, MXC had the most prominent effect on elongating the glandular ducts while GE enhanced the ductile branching. Combined exposure to GE and MXC resulted in substantial lobulo-alveolar growth of the mammary gland epithelium in male offspring. Such lobular structures are normally seen in postpubertal or early pregnant female rats. Immunostaining for proliferating cell nuclear antigen revealed a high percentage of immunopositive cells in the mammary epithelia of the males exposed to MXC and GE (800 ppm) compared to the controls.

In a follow-up study designed to evaluate male mammary responses to genistein and MXC at the *adult* stage, Wang *et al.* (2006) fed pregnant Sprague-Dawley rats (n=10 dams/group) a soy-and alfalfa-free diet containing different combinations of genistein (GE) (300 and 800 ppm) and/or MXC (800 ppm) from GD0, continuing through pregnancy and lactation, until PND 22, when the offspring were weaned. Following

weaning, the male offspring were fed the same treatment diets as their respective maternal groups. On PND 90, all rats were killed. Body weights/estimated doses were not reported in this follow-up study. The study authors reference the earlier study by You *et al.* (2002) described above. Table 6 provides estimated dose levels for both studies.

Both sides of the inguinal mammary glands from one male and one female pup in each of the 10 litters were removed at necropsy on PND 90 for evaluation of glandular development. One gland in each animal (4 animals/treatment group) was used for whole-mount preparation, and the other was used for histological section preparation; additional samples from other litters were also prepared for histological sections. Clontech Atlas Rat Toxicology 1.2 arrays (Clontech, Palo Alto, CA) were used to determine gene expression in the mammary gland tissue samples obtained from adult male rat offspring. Three individual rats were used in each of the four treatment groups (control, MXC 800 ppm, GE 800 ppm, and a combination of MXC 800 ppm and GE 800 ppm). Relative levels of gene expression were derived by comparing the group means (n=3) of the normalized intensity values in the GE, MXC, and GE+MXC to that of the control group. The S-Plus statistical program (Insightful, Seattle, WA) was also utilized in analyzing gene expression. The statistical outcomes of all tests were used to produce volcano plots classifying genes that were either up-regulated or down-regulated with statistical significance at the level of p=0.05.

The inguinal mammary gland of male offspring exhibited significant morphological alterations in the groups treated with GE, MXC, or their combinations GE+MXC compared to the control. MXC (800 ppm) exposure led to ductal elongation and lobular enlargement, whereas GE exposure (300 and 800 ppm) caused lobular enlargement and epithelial proliferation. Combining the two treatments caused prominent proliferation of both ducts and alveoli, changes not seen in normal male glands (*i.e.*, lobuloalveolar morphology normally seen only in differentiated female rat gland).

Latchoumycandane *et al*. (2002) exposed adult male Wistar rats (n= 4/group) to MXC at oral doses of 0, 50, 100 or 200 mg/kg-day for 1, 4 or 7 days. Body weights and weights of the testis, liver, and kidney did not show any significant changes in MXC-treated rats. The weight of the epididymis, seminal vesicles, and ventral prostate as well as epididymal sperm counts decreased after 50, 100 or 200 mg/kg-day MXC for 7 days, but remained unchanged after shorter courses of treatment. Weight changes in accessory sex organs were significantly decreased relative to controls ($p<0.05$) after 7 days at all exposure levels. Epididymal sperm motility was significantly decreased in a dose-dependent manner in the animals treated with MXC for 4 or 7 days. MXC administration at all levels for 4 or 7 days significantly decreased the activities of the antioxidant enzymes catalase, superoxide dismutase, glutathione reductase and glutathione peroxidase, while the levels of hydrogen peroxide and lipid peroxidation increased in a dose-dependent manner in epididymal sperm, and in the epididymis after 4 or 7 days of treatment. Co-administration of vitamin E (20 mg/kg-day) and MXC (200 mg/kg-day) for 7 days prevented MXC-associated changes in epididymal sperm counts and motility. The LOAEL for this study is the lowest dose tested, 50 mg/kg-day. Effects at this dose level included significant decrease in weights of accessory sex organs after treatment for 7 days, significant decrease in epididymal sperm motility after 4 or 7 days of treatment, and significant reduction in antioxidant activity after 4 or 7 days of treatment.

Immunotoxicity

Among the systems known to be sex differentiated, to contain abundant estrogen receptors, and to show dramatic changes at the time of puberty are the immuno-hematological and skeletal systems. Golub *et al.* (2004b) examined the effects on immune, hematologic and bone mass parameters in rhesus monkeys following oral exposure to MXC. Rhesus monkeys are a standard model for human health research. Like humans, female monkeys have a long and complex period of maturation during adolescence. Prepubertal female Rhesus monkeys (n=8/group) received daily oral doses of exogenous estrogen as 0.5 mg/kg diethylstilbestrol (DES) or 25 or 50 mg/kg MXC in the peripubertal period (six months before and after the expected age at menarche). Treatment with MXC had effects on both immunohematology and bone growth. MXC treatment apparently had an early effect on electrolyte balance; sodium, chloride, and total bicarbonate (TCO_2) concentrations were significantly higher in serum of MXC-treated monkeys than in controls three months after initiation of treatment. This difference did not appear at later sampling times, and TCO_2 was slightly lower in the MXC25 group at the end of treatment. Serum lipids demonstrated an estrogenic effect; a significant rise in triglycerides was seen in the DES and MXC25 groups at the end of treatment. The CD4+/CD29+ T-lymphocyte population was reduced in absolute numbers and as a percent of the lymphocytes only in the MXC50 group at the end of treatment, but recovered at the next sampling period. Cytokine assays (IL-4, IL-10), intended to probe T-helper TH1/TH2 activity, did not demonstrate clear differences among treatment groups, but showed a marginally significant greater ratio of IL-4 to IL-10 in the MXC25 group than in controls ($p = 0.05$) at the end of treatment. DES greatly decreased blood phosphorus, calcium, and alkaline phosphatase, and caused a significant decrease in bone mass, as determined with an X-ray densitometer. Interestingly the MXC50 group showed a significant decrease in bone mineral density in the femur neck and global proximal femur despite showing no decrease in phosphorus, calcium, and alkaline phosphatase. The authors concluded that disruption in these systems during adolescence could affect later risk for diseases such as osteoporosis, heart disease, and autoimmune disease.

Chapin *et al.* (1997) gavaged pregnant Sprague-Dawley rats (12/group) with MXC at 5, 50 or 150 mg/kg-day on GD14 through PND7, and then their pups (4-6/sex/litter) with the same doses until PND42. Live births were reduced at 150 mg/kg-day. Immune system evaluations of the pups at eight to nine weeks of age consisted of splenocyte mitogen LP responses, natural killer cell activity, plaque-forming cells (PFC) antibody response to sheep red blood cells (SRBCs), and flow cytometry phenotypic analysis of splenic lymphocytes. The PFC response to SRBCs was significantly suppressed by 35 and 42 percent in males exposed to 5 and 50 mg MXC/kg-day, respectively, but no suppression was observed in females. There was also a significant decrease in PFC/spleen in males. The reduction in live births in the high-dose group resulted in an insufficient number of males for evaluation at this dose. The male data indicate that the T cell-dependent antibody response to SRBCs was suppressed three weeks after the final exposure to MXC. The data suggest that a dose lower than 5 mg MXC/kg may also result in immunosuppression (LOAEL of 5 mg/kg-day in males).

Neurotoxicity

Environmental chemicals with estrogenic activity can potentially affect a number of central nervous system (CNS) functions. There is concern that estrogenic endocrine disruptors might interfere with development of the reproductive tract and other organ systems that mature under the influence of gonadal hormones, including the brain. The frontal cortex is of particular interest because it is one of the last areas of the brain to mature, and this maturation continues throughout adolescence. Prefrontal cortex has been proposed as an estrogen target tissue related to cognitive function (Berman *et al*., 1997; Duff and Hampson, 2000; Keenan *et al*., 2001).

Perinatal exposures to xenoestrogens, including MXC, have been shown to affect complex neural processes such as pain when they occur during critical stages of CNS development (Ceccarelli *et al*., 2009). Adult female rats exposed prenatally to xenoestrogens MXC or ethynylestradiol (EE) showed greater behavioral responses to a persistent painful stimulation than control rats. The authors concluded that this is evidence of the long-lasting effects of these estrogens on complex processes that involve the pain circuits. In this study, Sprague-Dawley female rats (n=51) were exposed either prenatally or postnatally to 20 µg/kg-day MXC (from GD 5 until post-natal day 21). They were subjected to a series of tests at 1-week intervals starting from 23 weeks of age. Object recognition, Plantar, and Formalin tests were carried out to evaluate the effects of these compounds on integrated functions such as memory and pain. The Formalin test is a well-known model of persistent pain which measures licking duration, flexing, and paw-jerk in response to dilute formalin being injected sc in the right dorsal hind paw. Testosterone and estradiol plasma levels were determined by radioimmunoassay (RIA). The results of the Object Recognition and Plantar tests did not differ among groups. In the Formalin test, flexing duration was higher in the prenatally-exposed MXC and EE groups than in the MXC and EE postnatal exposure and control (oil-exposed) groups. Since flexing duration is elevated by prenatal treatment, the authors hypothesize that exposure to MXC (and EE) during fetal life affects the neuronal circuits in which estrogen receptors are present and induces long-lasting effects on pain modulation.

Large doses of MXC, 2,500 mg/kg or more administered orally to rats, decreased locomotor activity and caused tremors (Cannon Laboratories, 1976). In dogs, 1,000-4,000 mg/kg-day MXC orally for 8-24 weeks produced dose-dependent apprehension, nervousness, increased salivation, tremors, convulsions, and death (Tegeris *et al*., 1966). Inhibiting metabolism of MXC appeared to increase the acute tremors, suggesting that this effect is due to the intact chemical. This is consistent with the observation that DDT, which is a close structural analogue, but is very slowly metabolized, produces similar neurological signs (ATSDR, 1994). An increased incidence of hunched posture and rough fur was reported in rats exposed to 22-69 mg/kg-day MXC in feed for 78 weeks (NCI, 1978). No changes in brain weight or histopathology were noted in rats or mice chronically exposed to 69 and 454 mg/kg-day MXC, respectively (NCI, 1978).

Exposure to MXC has also produced behavioral changes in animals consistent with its estrogenic actions (Gray *et al*., 1988), such as increased wheel-running activity and receptivity to mating.

Golub *et al.* (2004a) treated prepubertal female Rhesus monkeys (n=8/group) with daily oral doses of 25 or 50 mg/kg MXC or 0.5 mg/kg diethylstilbestrol (DES) for six months before and after the anticipated age of menarche (these are the same animals described regarding other measurements in Golub *et al.*, 2004b). Behavior was assessed during and for nine months after dosing. Visual pattern discrimination and visual recognition memory were assessed during and after treatment. Spatial working memory was assessed six months after cessation of dosing to examine potential long-term effects on brain maturation. In addition, spontaneous motor activity and sleep-wake patterns, both of which are influenced by puberty in monkeys, were assessed during and after dosing. And finally, the auditory brainstem response (ABR), which demonstrates sensitivity to estrogen in women, was measured 18 months after dosing was completed. Despite the lower overall estrogenic potential of MXC relative to DES, the MXC50 treatment was more disruptive to behavior. All three treatment groups (MXC and DES) performed more poorly than controls on the visual pattern discrimination trials; the MXC50 group had consistently poorer performance. Spatial working memory also showed acquisition deficits and possible working memory deficits in the MXC50 group. The authors conclude that differential effects of the two agents at the estrogen receptor subtypes (ERα and ERß) may be related to the observed differences in behavioral outcomes.

Palanza *et al.* (2002) exposed pregnant CD-1 female mice to low, potentially environmentally relevant doses of MXC and studied the effects on behavior of both dams and offspring. From GD11 to 17, 18-21 female mice/group spontaneously drank MXC in corn oil to receive doses of 0, 20, 200 or 2,000 µg MXC/kg-day. The methods state that mice were treated every second day, but doses are reported only on a daily basis, which lends some confusion as to the actual treatment. Maternal behavior was examined from post-partum days 2 to 15. The offspring were subjected to a series of behavioral tests at different ages.

MXC treatment during pregnancy appeared to produce slight changes in maternal behavior: dams exposed to the lowest dose of MXC, 20 µg/kg, spent less time nursing the pups, and more time eating, resting, and self-grooming than control dams. Dams of the MXC 200 and 2,000 µg/kg groups showed similar, mostly non-significant trends; the three MXC treatment groups do not appear to differ significantly from each other. Prenatal MXC treatment affected behavioral responses to novelty in both sexes at periadolescence. The onset of male intrasex aggression (all males tested, by sibling group) was delayed in males prenatally exposed only to the lowest dose of MXC. MXC tended to decrease the sexual dimorphism in activity levels in the novel environment, mostly at the lowest dose. A sex difference was observed in the control group, with males being significantly more active in the open field than females. MXC-exposed females, but not males, showed increased exploration in an unfamiliar open field. The 20 µg/kg-day dose to dams represents a LOAEL for these studies. However, the authors conclude that "the concept of threshold dose cannot be applied to EDCs since they mimic or antagonize the actions of endogenous molecules important to development." That is, the homeostatic regulatory system is pushed up or down by competing receptor agonists and antagonists, proportional to net receptor activity.

Perinatal exposure to a very low-dose of MXC has been shown to have long-term consequences on neurobehavioral development (sexual differentiation) in both male and

female mice. The last days of gestation and the first week after birth is a critical period of brain development in rats and mice. Gioiosa *et al.* (2007) orally dosed pregnant CD-1 mice from GD11 to post partum day 8 with 0 or 20 µg/kg-day MXC dissolved in corn oil (n = 15 controls, 9 treated). One pup of each sex from each litter (13 males and 14 female controls, 12 males and 12 female treated) were examined at various ages (before and after puberty) in three behavioral tests designed to test explorative and emotional behaviors. As adolescents, mice underwent a novelty test which measures impulsivity and novelty seeking levels. As adults, mice were tested in a free-exploratory open-field, which allows for measurements of anxiety and activity levels, as well as their propensity to explore. In a third test, mice were evaluated on the elevated plus maze test, a traditional paradigm to test anxiety levels in mice and rats. These paradigms show sex differences in rodents, including mice. Behavioral data were collected by a trained observer, blind to the experimental groups.

Male and female control animals showed characteristic behavioral sex differences, and differed on a number of behavioral responses at both ages and in all experimental paradigms. Mice exposed to MXC showed decreased or no sex differences (*i.e.*, elimination of sexual dimorphism). In all three test paradigms, behavior of MXC-exposed mice was significantly affected by treatment (male versus female) on some of the behavioral measures rated. Female mice appeared more sensitive than males to the neurobehavioral effects of MXC; that is, the activity of males was not significantly different from controls on any of the tests, while the activity of females differed significantly from controls in five of the 14 test results presented. The lack of effects on males in the plus maze at 20 µg/kg-day differs from the slight decrease in activity observed in the same laboratory earlier (Palanza *et al.* (2002). The behavior of exposed females more closely resembled that of control males than control females. These findings are consistent with an estrogenic action of MXC, and possible defeminization or masculinization effects, although the lack of a positive control makes it more difficult to categorize potential mechanism(s) of action. The authors conclude that these findings should be cause for public health concern, confirming that low-dose exposure to a weak estrogenic agent during brain sexual differentiation can result in persistent impairment of sexually differentiated adult behaviors. These findings agree with other studies that show that estrogenic endocrine disruptors such as MXC interfere with the processes of sexual differentiation of brain and behavior in a number of animal models. The LOAEL for this effect on female offspring is 20 µg/kg-day, administered to the dams during the critical perinatal period. The dose of MXC is said by the authors to be within the range of human exposure; however, current U.S. exposures to this cancelled pesticide are several orders of magnitude lower, as discussed above in the section on Environmental Occurrence and Human Exposure.

Chronic Toxicity

Several chronic studies have been carried out on MXC, although none of these meet current U.S. EPA guidelines (Haag *et al*., 1950; Hodge *et al*., 1952; Deichmann *et al*., 1967; NCI, 1978). Decreased body weight gain was observed in rats at 69 mg/kg-day (NCI, 1978) or 125 mg/kg-day (Haag *et al*., 1950) and in mice at 454 mg/kg-day (NCI, 1978).

Unlike many other polycyclic aromatic hydrocarbons, little liver enzyme induction occurs with chronic exposures to MXC, apparently because of its rapid metabolism. However, significant liver enzyme induction does occur with multiple daily doses of MXC, which will further increase its cytochrome P_{450}-mediated metabolism (Li *et al*., 1995). Traditional microsomal enzyme inducers such as phenobarbital can also induce MXC metabolism (Stresser *et al*., 1996).

The most significant effects of repeated doses of MXC are on reproductive tissues. Chronic effects can occur after subacute exposures to MXC during critical developmental stages. Perinatal administration of moderate doses of MXC causes persistent stimulation of ovarian/uterine development, well into adulthood. High doses inhibit development, which is similar to the effects of other estrogens, according to Eroschenko *et al.* (1995). Alteration of adult behaviors in male mice by prenatal exposures to low doses of MXC and other estrogenic chemicals has also been reported (vom Saal *et al*., 1995). Administration of a few high doses of MXC to female mice during one pregnancy also appeared to alter the vaginal development of female offspring in a subsequent pregnancy (Swartz and Corkern, 1992).

Chronic administration of MXC disrupts sex-hormone sensitive systems by direct action of the metabolites on the end organs as well as effects on the feedback loops (Cummings, 1997). Uterine weights increase because the uterus responds directly to estrogens (Tullner, 1961), while testicular weights decrease (Hodge *et al*., 1950; Bal, 1984), presumably because of indirect effects of the metabolites on androgenic tissues. Subchronic or chronic administration of MXC will thus impair reproduction in both males and females (Harris *et al*., 1974; Bal, 1984; Cummings and Gray, 1989; Gray *et al*., 1989). Chronic administration does not result in accumulation of MXC or its metabolites; enhanced toxicity compared to subacute administration during critical developmental periods has not been observed.

Carcinogenicity

Carcinogenicity studies on MXC are inadequate by present standards (such as U.S. EPA guidelines and NTP protocols), and existing data show little evidence of effects. Hodge *et al.* (1952) reported no significant increase in tumors in rats at daily oral doses up to 80 mg/kg for two years. Deichmann *et al.* (1967) found no increase in tumors in rats at 50 mg/kg-day in feed for two years. The NCI (1978) also reported that MXC was not carcinogenic in rats fed up to 69 mg/kg-day and in mice fed up to 454 mg/kg-day for 78 weeks. However, Reuber evaluated several chronic studies, including some unpublished FDA data, and concluded that MXC is carcinogenic. His judgment was that MXC produced liver tumors in mice, rats, and possibly dogs, testicular tumors in male mice, bone cancer in female mice, and ovarian tumors in female rats (Reuber, 1979a,b, 1980). The U.S. EPA (1987b) reevaluated these data and concluded that Reuber's analyses involved inappropriate use of control data and questionable histopathological interpretations. Both the U.S. EPA and the International Agency for Research on Cancer (IARC) have subsequently rated MXC as not classified as to human carcinogenicity (IARC, 1987; U.S. EPA, 2010).

Further evidence on potential carcinogenicity of MXC includes some positive mutagenicity results (Mitchell *et al*., 1988; Oberley *et al*., 1993), formation of reactive intermediates which produce covalently bound protein adducts (Bulger *et al*., 1983), structure-activity correlations based on the carcinogenicity of related weakly estrogenic compounds such as DDT and DDE, and evidence that estrogens are risk factors in testicular tumors (McLachlan, 1998). Estrogenic effects on testes during critical developmental periods are considered as a potential cause of cancer. Metabolic cooperation in Chinese hamster cells was inhibited by MXC. This was similar to the effects of DDT and its analogues, and is a possible indicator of promoter activity (Kurata *et al.,* 1982). However, dermal administration of MXC neither induces nor promotes the formation of skin tumors (Dwivedi and Tabbert, 1994). WHO (2004) concluded that MXC "may be a tumor promoter" and applied an uncertainty factor of 10 in the risk assessment for MXC in drinking water for "concern for threshold carcinogenicity and the limited database."

Although the reproductive system is recognized as the major target organ of MXC, the potential exists for disrupting all other endocrine organs having estrogen receptors. 17ß-Estradiol 3-benzoate (EB), a typical endocrine-disrupting chemical, enhances thyroid tumorigenesis while increasing estrogen receptors in female ovariectomized rats (Ito *et al.,* 1995). Takagi *et al.* (2002) evaluated the ability of several endocrine disrupting chemicals (EDCs), including MXC and EB, to affect the development of thyroid proliferative lesions. Six-week old female castrated F344 rats were first given a single subcutaneous injection of 2,000 mg/kg of *N*-bis(2-hydroxypropyl)nitrosamine, a known initiator of thyroid carcinogenesis. From one week later, they received diets with no supplement (control), pellets with 0.5 mg EB, or a diet mixed with 1,000 ppm MXC or 10,000 ppm bisphenol A (BPA) for 20 weeks (n=12/group). Additional groups were administered 200 ppm sulfadimethoxine (SDM) in the drinking water simultaneously with the control, EB, MXC or BPA treatments. SDM has been reported to induce thyroid adenomas and carcinomas when administered in drinking water. Doses for MXC and BPA were selected based on the maximum tolerated dose in long-term toxicity studies. A dietary level of 1,000 ppm MXC would provide a dose of about 50 mg/kg-day, assuming a feed consumption rate of 5 g/100 g body weight.

MXC treatment had significant effects on body weight, various organ weights, and thyroid hormone levels. However, MXC did not promote thyroid proliferative lesions in rats, nor did BPA, another weak estrogenic agent. Only EB, with strong estrogenic activity, induced thyroid follicular cell hyperplasias, adenomas and/or carcinomas in the EB + SDM group. Body weights in the EB and EDC treated groups were significantly lower than in controls, with or without SDM treatment. In accordance with the decreased body weights, the absolute liver weights were significantly decreased, while the relative weights of the pituitary, thyroid, and uterus were significantly increased in the MXC treatment groups, with or without SDM treatment. Serum T3 levels in the EB alone group were significantly increased, while those of the MXC + SDM group were significantly decreased compared with controls. Serum T4 levels in both the EB and MXC treated groups were significantly decreased, independent of SDM treatment. The T4 levels of the MXC + SDM, BPA + SDM and EB + SDM groups were significantly decreased compared with the groups without SDM. The authors suggest that the decrease

in serum T4 levels in the MXC alone and MXC + SDM groups might be related to the potential of MXC to inhibit hepatic iodothyronine 5-monodeiodinase (Zhou *et al*., 1995).

Toxicological Effects in Humans

Acute Toxicity

Several reports of toxic effects in humans acutely exposed to MXC are available, although all involve single cases, and most entail exposure to pesticide mixtures. Zeim (1982) reported delayed adverse effects in a 49-year-old male exposed by inhalation to a mixture of MXC and captan (a fungicide often used in mixtures with insecticides on fruit trees). The subject died six months after the exposure due to aplastic anemia. Exposure levels are unknown and the relationship of the effect to the MXC exposure is uncertain.

Harell *et al*. (1978) reported neurological effects after exposure for 15 to 20 minutes to a pesticide mixture containing 15 percent MXC and 7.5 percent malathion. The 21-year old male noted blurred vision and nausea 8-9 hours after exposure, followed by severe abdominal cramps and diarrhea that required hospital admission 36 hours after exposure, followed by dizziness and complete deafness 4 days later, accompanied by several sensory and motor neurological impairments such as limb paresthesia. These effects persisted for at least six years. The authors postulated that the effects could be due to a deficiency of a malathion-metabolizing enzyme. There are no other reports of such effects from malathion, MXC, or a mixture of the two, so the relationship of the effects to MXC exposure is unknown.

A 62-year old man attempted suicide by ingesting a commercial product containing 120 mg/L MXC as the active ingredient (Thompson and Vorster, 2000). The man was suspected of ingesting 100-150 mL of product. Upon admission to the hospital, the man was not responsive to pain or verbal stimuli. His skin was pale, and he was diaphoretic. A strong chemical odor was present. Initial diagnosis revealed a blood pressure of 58/40 and a pulse rate of 88 beats/minute. A serum sample collected at the time of admission contained 0.67 µg/mL MXC. Treatment at the hospital included intravenous Ringer's Lactate 02. Once the patient responded to treatment, his blood pressure was found to be 110/70. Neurological activity included hypertonic lower extremities. The patient appeared to have fully recovered by the time he was discharged from the hospital (no recovery timeline or additional details were provided).

Subchronic Toxicity

A single study of subchronic administration of MXC has been conducted in humans. In this study, oral doses of 2 mg/kg-day of MXC (the only dose level studied) for seven days/week for six weeks had no reported adverse effects in either men or women (Stein, 1968; Coulston and Serrone, 1969). Blood studies and bone marrow and liver biopsies revealed no changes attributable to MXC. Two mg/kg-day was considered to be a NOAEL (ATSDR, 1994).

Genetic Toxicity

MXC was found not to increase single-stranded DNA breaks in human (or rat) testicular cells *in vitro*, in an analysis of the ability of several chemicals to induce genetic damage (Bjorge *et al*., 1996).

Developmental and Reproductive Toxicity

Effects of MXC on estrogen receptors were shown to be similar in experimental animals and human tissues (Shelby *et al*., 1996). MXC was active in a yeast human estrogen receptor assay, which was inferred as evidence for metabolism of MXC to the phenolic metabolite as well as competence in binding of the metabolite to the human receptor (Odum *et al*., 1997; Gaido *et al*., 1997). Histopathologic changes were not found in the testes of men experimentally exposed for 6 weeks to up to 2 mg/kg of MXC, nor were there changes in menstrual cycles of women after the same dosing regimen (Stein, 1968; Coulston and Serrone, 1969). By analogy with effects observed in animals and effects caused by other estrogenic chemicals such as diethylstilbestrol, it is likely that effects would be observed at lower doses if humans were treated during a more sensitive period, such as during the development and maturation of reproductive organs (see Chapin *et al*., 1997).

Immunotoxicity

No information is available on immunological effects of MXC in humans.

Neurotoxicity

The acute excitatory effect of MXC associated with DDT-like stimulation at sodium channels should be applicable to humans. However, no reports of human exposures to high doses where this effect would be expected are available. The only report concerning potential neurotoxic effects of MXC is the report of Harrell *et al*. (1978) mentioned above, in which persistent deafness and severe neurological changes occurred in an adult male beginning several days after a single exposure to a mixture of MXC and malathion. The relationship of MXC to these effects is not known.

Chronic Toxicity/Carcinogenicity

An epidemiological study of men in Minnesota and Iowa suggested an association between leukemia and farming (Brown *et al*., 1990). In this study, there were positive correlations with odds ratios (ORs) of 2.0 or more for exposure to several agricultural pesticides including three organophosphates (adjusted OR 2.0 to 11.1), pyrethrins (OR 3.7), and MXC (OR 2.2). For MXC, this represented 11 cases of leukemia among 578 farmers with occupational exposure to MXC versus 16 cases out of 1,245 controls with no known exposure. The statistically significant OR of 2.2 for MXC incorporated adjustments for vital status, age, state, tobacco use, family history of lymphopoietic cancer, high-risk occupations and high risk exposures. However, firm conclusions as to a

relationship between MXC and leukemia are not possible on the basis of this single study with multiple exposures and risk factors.

Another epidemiological study that examined the risk of childhood leukemia (the most common childhood cancer) and residential exposure to persistent organochlorine chemicals, including MXC, found *no* significant positive associations for MXC (Ward *et al.*, 2009). The population-based case-control study was conducted in 35 counties in northern and central California from 2001-2006. The study included 184 acute lymphocytic leukemia (ALL) cases 0-7 years of age and 212 birth certificate controls matched to cases by birth date, sex, race and Hispanic ethnicity. The study authors collected carpet dust samples from the room where the child spent the most time before diagnosis. MXC was detected in dust samples taken from 34 case households and 50 control households. (Detection of any polychlorinated biphenyl (PCB) congener in the dust was associated with a 2-fold increased risk of ALL).

No studies are available pertinent to other possible effects of chronic MXC exposure, including the presumed critical effect, endocrine system changes. The widely discussed potential effects of environmental estrogens on reproductive function (Toppari *et al*., 1996; U.S. EPA, 1997; Safe *et al*., 1998; Cheek *et al*., 1998, Welshons *et al.*, 2003) cannot be specifically associated with MXC from environmental exposures because of its short biological half-life and low or undetectable concentrations in the environment.

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Effects

Oral exposure to MXC can produce gross and histopathological changes, and affect development and function in the male and female reproductive system. The lowest-dose effects have been observed on prostate and liver weights, with treatments during the perinatal period. Judy *et al.* (1999) observed endocrine disruption in adult offspring by MXC in the low µg/kg range of maternal exposure. Pregnant CF-1 mice received 20 or 2,000 µg/kg MXC in 30 µL corn oil daily from GD 11 to GD 17. The fetal exposure to MXC resulted in a significant increase in *adult* prostate and seminal vesicle weights, which indicates a long-term effect in both prostate and seminal vesicles. Both 20 and 2,000 µg/kg MXC resulted in a lower liver weight relative to controls. These data are presented in Table 5.

These data are consistent with a number of other studies showing LOAELs of ~20 µg/kg following perinatal treatment with MXC (Gioiosa *et al*., 2007; Palanza *et al*., 2002; vom Saal *et al*., 1995), summarized in Table 7. The perinatal period of sexual differentiation is the exposure window for these studies. The perinatal period in the mouse corresponds to sexual differentiation in the human fetus during the second trimester. Because hormones are biologically active at very low levels in the body, estrogenic agents such as MXC that mimic these natural hormones may also act at low exposure levels, potentially much lower than required for acute toxicity.

Table 7. Lowest-Dose Methoxychlor Effects in Animal Studies

Study	Animal Model	Route and Time of Exposure	Doses (maternal mg/kg)	NOAEL and/or LOAEL	Significant Adverse Effect(s)
Vom Saal *et al*., 1995	Pregnant CF-1 mice	Oral, GD 11-17	0, 0.02, 2, 100	20 ug/kg-day (LOAEL)	Alterations in social-sexual behavior in male offspring, measured as increased urine marking
Judy *et al*., 1999	Pregnant CF-1 mice	Oral, GD 11-17	0, 0.02, 2.0	20 µg/kg-day (LOAEL)	Increase in relative prostate (both dose levels) and seminal vesicle weight (high dose only), decrease in relative liver wt (both doses) in adult male offspring
Palanza *et al*., 2002	Pregnant CF-1 mice	Oral, GD 11-17	0, 0.02, 0.2, 2.0	20 µg/kg-day (LOAEL)	Behavioral effects in offspring (decreased sexual dimorphism in activity level in males, increased exploration in unfamiliar open field in female)
Gioiosa *et al*., 2007	Pregnant CF-1 mice	Oral, GD 11 to PPD 8	0, 0.02	20 µg/kg-day (LOAEL)	Behavioral effects on sexually differentiated behavior in adult female offspring

GD = gestational day; PPD = postpartum day

The PHG is derived from the LOAEL of 20 µg/kg-day for developmental effects on reproductive system parameters in an animal study of Judy *et al.* (1999). The adult sexual-related behavioral effects reported at the same dose by Vom Saal *et al.* (1995, Palanza *et al.* (2002), and Gioiosa *et al.* (2007) are also very notable; we have chosen the organ weight changes as the critical effects merely because these are common, well-known endpoints.

Carcinogenic Effects

Carcinogenicity studies on MXC are inadequate by present standards (such as U.S. EPA guidelines and NTP protocols), and existing data show little evidence of effects. Both the U.S. EPA and the International Agency for Research on Cancer (IARC) have judged MXC to be not classified as to human carcinogenicity (IARC, 1987; U.S. EPA, 2010).

The U.S. EPA (2010) carcinogenicity file in IRIS is identified as last revised in1990, but also notes that "A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the cancer assessment for Methoxychlor conducted in August 2003 did not identify any critical new studies."

CALCULATION OF PHG

Noncarcinogenic Effects

For estimation of a health-protective concentration of a chemical in drinking water, an acceptable daily dose of the chemical from all sources will first be calculated. This involves incorporation of appropriate estimates of uncertainty in the extrapolation of the critical toxic dose from human or animal studies to the estimation of a lifetime daily dose for methoxychlor that is unlikely to result in any toxic effects. For this purpose, the following equation will be used:

$$\text{ADD} = \frac{\text{NOAEL/LOAEL in mg/kg-day}}{\text{UF}}$$

where,

ADD = an estimate of the maximum daily dose which can be consumed by humans for an entire lifetime without toxic effects;

NOAEL/LOAEL = no-observed-adverse-effect level or lowest-observed-adverse-effect level in the critical study;

UF = uncertainty factor, which typically includes factors of 10 for interspecies and intraspecies extrapolations, from a LOAEL to a NOAEL, or from subchronic to chronic exposure, to a maximum of 3,000.

Using the lowest LOAEL of 20 µg/kg-day from the Judy *et al*. (1999) study, we applied an uncertainty factor of 10 for cross-species extrapolation, 10 for human variability, and 10 for extrapolation from a LOAEL to a NOAEL, for a combined uncertainty factor of 1,000. This combined uncertainty factor should be large enough to account for any sensitive human populations and potential interactions with other estrogenic chemicals. Thus,

$$\text{ADD} = \frac{0.02 \text{ mg/kg-day}}{1{,}000} = 2\text{x}10^{-5} \text{ mg/kg-day (0.02 µg/kg-day)}$$

Calculation of concentrations of chemical contaminants in drinking water associated with negligible risks must take into account the toxicity of the chemical and the potential exposure of individuals using the water. Tap water is used directly as drinking water and for preparing foods and beverages. It is also used for bathing or showering, flushing toilets, washing dishes and clothes, and other household uses that may result in dermal and inhalation exposures to chemical contaminants. However, because MXC is non-volatile, secondary inhalation exposures are expected to be negligible.

Other sources of exposure to MXC include food (pesticide residues) and possible occupational or household uses. Although MXC is no longer registered as a pesticide in the U.S, it may still be used in other countries. It has been detected more often in food samples than in drinking water supplies. We consider food to represent the largest likely exposure medium. A default relative source contribution of 20 percent for drinking water is therefore used in calculation of the PHG.

Drinking water consumption is estimated for this purpose based on the upper 95th percentile of consumption of a community water supply (tap water), as reported by the U.S. EPA (2004b) based on water consumption in a nationwide survey. The tap water consumption rate for pregnant women, 0.043 L/kg-day, will be used because the exposures leading to the critical developmental effects were during pregnancy.

Calculation of the public health-protective concentration (C, in mg/L) for MXC in drinking water will use the following equation:

$$C = \frac{\text{ADD mg/kg-day x RSC}}{\text{L/kg-day}}$$

where,

RSC = relative source contribution (usually 20 to 80 percent, expressed as 0.20 to 0.80);

L/kg-day = upper 95th percentile of daily water consumption for pregnant women, the critical population in this case.

Thus,

$$C = \frac{2\times10^{-5}\ \text{mg/kg-day x } 0.2}{0.043\ \text{L/kg-day}} = 9.3\times10^{-5}\ \text{mg/L}\ (0.09\ \mu\text{g/L or ppb})$$

A PHG of 0.09 µg/L is therefore determined for MXC in drinking water based on the LOAEL of 20 µg/kg-day for developmental effects on the male rat reproductive system (increased relative prostate weight and decreased relative liver weight). This value is judged to be adequate to protect sensitive populations, including pregnant women, infants, and children, from all adverse effects of MXC, including the critical estrogenic effects of MXC in drinking water.

RISK CHARACTERIZATION

Exposure to multiple estrogenic chemicals in our environment has been a matter of much recent discussion and concern (Shelby *et al*., 1996; Toppari *et al*., 1996; U.S. EPA, 1997; DeRosa *et al*., 1998; Safe, 1998; Cheek *et al*., 1998, Welshons *et al*., 2003). MXC is listed as a persistent, bioaccumulative and toxic (PBT) chemical by the U.S. EPA Toxics Release Inventory (TRI) program. Several studies make clear that MXC, mainly through its demethylated metabolites, can bind to intracellular human estrogen receptors

(Cummins, 1997; Danzo, 1997, Gaido *et al*., 1997, 1999, 2000). Thus it has the potential to be a problem, although its short environmental and *in vivo* half-life will minimize both exposure and effects, compared to other structurally similar halogenated hydrocarbons.

The primary source of uncertainty in developing the PHG for MXC in drinking water is the question of human sensitivity to the potential endocrine-disruptive effects. Because many endocrine functions are feedback-regulated, exposures to small amounts of estrogenic chemicals may simply be accommodated by normal homeostatic mechanisms (U.S. EPA, 1997). On the other hand, in animals, exposure to very low doses of MXC (1.0 µg/day) has been shown to disrupt systems that differentiate under endocrine control (vom Saal *et al*., 1995). Studies are inadequate to fully characterize sensitive developmental periods and corresponding effective estrogenic doses and concentrations in humans. Therefore it is not clear what dose-equivalent of an estrogenic chemical would be physiologically significant, particularly in the presence of other environmental estrogens in food or water (U.S. EPA, 1997; Safe, 1998; Cheek *et al*., 1998; Welshons *et al*., 2003). However, the animal studies appear adequate to document the effects, mechanism of action, and potency of MXC in animals, including the potential for sensitive developmental periods. The period of fetal sexual differentiation is critical in development. During this time, organs are particularly susceptible to the disruptive effects of chemicals that have hormonal or antihormonal activity. This is taken into account in the choice of the critical study (Judy *et al*., 1999) and application of uncertainty factors.

Oral ingestion through food would be expected to be the major route (if any) of human exposure to MXC. Studies in animals have shown combination effects of genistein (an estrogenic isoflavone naturally present in soybeans and other legumes) and MXC on reproductive development of offspring of both sexes (You *et al*., 2002; Wang *et al*., 2006). Like MXC, genistein has a well-documented ability to bind to the estrogen receptor and exert estrogenic effects on reproductive development in animals (Casanova *et al.,* 1999). Dietary exposure to phytoestrogens is common for both humans and animals; exposures occur through regular dietary intake or through dietary supplements of phytoestrogens. Hence, individuals consuming a soybean-based diet with concomitant exposure to MXC residues in food/drinking water may be at increased risk from the adverse effects of these endocrine-disrupting chemicals, particularly during sensitive periods of development (e.g., perinatal and/or prepubertal periods).

An appropriate relative source contribution (RSC) to account for the potential multiple exposure routes is uncertain. We have used the default 0.2 because of inadequate data for a more precise calculation. OEHHA believes that the PHG level of 0.09 µg/L is adequate and appropriate to protect humans, including sensitive subpopulations, against adverse effects of MXC in drinking water.

OTHER REGULATORY STANDARDS

The U.S. EPA oral reference dose (RfD) for MXC is 0.005 mg/kg-day, as currently listed in IRIS (U.S. EPA, 2010), last updated 08/01/1991. The RfD is based on the Kincaid Enterprises study (1986) in rabbits, with a lowest effect level of 35.5 mg/kg-day, a NOEL

of 5.01 mg/kg-day, and a UF of 1,000. Confidence in the RfD is rated as low because of the lack of definitive chronic toxicity studies and the limitations of the developmental studies available at that time. WHO (2004) concluded that MXC "may be a tumor promoter" and applies an uncertainty factor of 10 in the risk assessment for MXC in drinking water for "concern for threshold carcinogenicity and the limited database."

The U.S. EPA MCL and MCLG for MXC in drinking water are both 0.04 mg/L (40 ppb) (U.S. EPA, 2009), and were originally finalized in 1991 (56 FR 3526, 01/30/91). The California MCL is 0.03 mg/L, decreased from 0.04 mg/L in June 12, 2003 in response to the 1999 PHG of 0.03 mg/L (DPH, 2009).

MXC is listed as a persistent, bioaccumulative and toxic (PBT) chemical by the U.S. EPA Toxics Release Inventory (TRI) program. MXC is not listed as a developmental or reproductive toxicant under California's Proposition 65. The ambient water quality criterion for human health (consumption of water plus organisms) is 0.1 mg/L (U.S. EPA, 1976; 2006). The chronic ambient water quality criterion for fresh and salt water for aquatic organisms is 3×10^{-5} mg/L (U.S. EPA, 2006). ATSDR's acute and subchronic oral maximum residue levels are 0.02 mg/kg-day (ATSDR, 1994). The most recent OEHHA risk assessment of MXC to develop a child-specific reference dose (chRD) for use in school site risk assessment (OEHHA, 2005) is 0.00002 mg/kg-day, based on the same study and approach used for the PHG, and is identical to the ADD calculated above.

REFERENCES

Akingbemi BT, Ge RS, Klinefelter GR *et al*. (2000). A metabolite of methoxychlor, 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane, reduces testosterone biosynthesis in rat Leydig cells through suppression of steady-state messenger ribonucleic acid levels of cholesterol side-chain cleavage enzyme. Biol Reprod 62:571-8.

Alworth LC, Howdeshell KL, Ruhlen RL, Day JK, Lubahn DB, Huang TH, Besch-Williford CL, vom Saal FS (2002). Uterine responsiveness to estradiol and DNA methylation are altered by fetal exposure to diethylstilbestrol and methoxychlor in CD-1 mice: effects of low versus high doses. Toxicol Appl Pharmacol 183:10-22.

Appel RJ, Eroschenko VP (1992). Passage of methoxychlor in milk and reproductive organs of nursing female mice; 1. Light and scanning electron microscopic observations. Reprod Toxicol 6(3):223-31.

Arnold SF, Klotz DM, Collins BM, Vonier PM, Guillette LJ, McLachlan JA (1996). Synergistic interactions of estrogen receptor with combinations of environmental chemicals. Science 272:1489-92.

ATSDR (1994). Toxicological profile for methoxychlor. U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, Atlanta, GA. TP-93/11.

ATSDR (2002). Toxicological profile for methoxychlor. U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, Atlanta, GA. Accessed at: http://www.atsdr.cdc.gov/toxprofiles/tp47.pdf.

Bal HS (1984). Effect of methoxychlor on reproductive systems of the rat. Proc Soc Exp Biol Med 176:187-96.

Berman KF, Schmidt DR, Rubinow MA *et al*. (1997). Modulation of cognition-specific cortical activity by gonadal steroids: a positron-emission tomography study in women. Proc Natl Acad Sci 94:8836-8841.

Bjorge C, Brunborg G, Wiger R, Holme JA, Scholz T, Dybing E, Soderlund EJ (1996). A comparative study of chemically induced DNA damage in isolated human and rat testicular cells. Reprod Toxicol 10(6):509-19.

Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM, Burmeister LF, Van Lier SF, Dick F (1990). Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Res 50(20):6585-91.

Bulger WH, Feil VJ, Kupfer D (1985). Role of hepatic monooxygenases in generating estrogenic metabolites from methoxychlor and from its identified contaminants. Mol Pharmacol 27:115-24.

Bulger WH, Kupfer D (1989). Characteristics of monooxygenase-mediated covalent binding of methoxychlor in human and rat liver microsomes. Drug Metab Dispos 17(5):487-94.

Bulger WH, Kupfer D (1990). Studies on the formation of methoxychlor-protein adduct in rat and human liver microsomes. Is demethylation of methoxychlor essential for cytochrome P450 catalyzed covalent binding? Biochem Pharmacol 40(5):937-45.

Bulger WH, Temple JE, Kupfer D (1983). Covalent binding of [^{14}C]methoxychlor metabolite(s) to rat liver microsomal components. Toxicol Appl Pharmacol 68(3):367-74.

Cannon Laboratories (1976). Acute oral toxicity in rats: Technical methoxychlor. Reading, PA. Unpublished study cited in ATSDR, 1994.

Casanova M, You L, Gaido W *et al.* (1999). Developmental effects of dietary phytoestrogens in Sprague-Dawley rats and interaction of genistein and daidzein with rat estrogen receptors α and ß *in vitro*. Toxicol Sci 51:236-244.

CCR (1998). California Code of Regulations Title 22, Article 5.5 Primary Standards – Organic Chemicals, Section 65555.

Ceccarelli I, Fiorenzani P, Della Seta D, Massafra C *et al.* (2009). Perinatal exposure to xenoestrogens affects pain in adult female rats. Neurotoxicol Teratol 31:203-9.

Chapin RE, Harris MW, Davis BJ, Ward SM, Wilson RE *et al*. (1997). The effects of perinatal/juvenile methoxychlor exposure on adult rat nervous, immune, and reproductive system function. Fund Appl Toxicol 40(1):138-57.

Cheek AO, Vonier PM, Oberdorster E, Burow BC, McLachlan JA (1998). Environmental signaling: a biological context for endocrine disruption. Environ Health Perspect 106, Suppl 1:5-10.

Coulston F, Serrone DM (1969). The comparative approach to the role of nonhuman primates in evaluation of drug toxicity in man: a review. Ann NY Acad Sci 162:681-704.

Culik R, Kaplan AM (1976). Teratogenic study in rats with ethane, 1,1,1-trichloro-2,2-bis(paramethoxyphenyl) (methoxychlor). Haskell Laboratory Report No. 648-76. Unpublished study cited in ATSDR, 1994.

Cummings AM (1997). Methoxychlor as a model for environmental estrogens. Crit Rev Toxicol 27(4):367-379.

Cummings AM, Gray LE (1989). Antifertility effect of methoxychlor in female rats: Dose and time-dependent blockade of pregnancy. Toxicol Appl Pharmacol 97:454-62.

Cummings AM, Perreault SD (1990). Methoxychlor accelerates embryo transport through the rat reproductive tract. Toxicol Appl Pharmacol 102:110-6.

Cummings AM, Laskey J (1993). Effect of methoxychlor on ovarian steroidogenesis: role in early pregnancy loss. Reprod Toxicol 7:17-23.

Cummings AM, Metcalf JM (1994). Mechanisms of the stimulation of rat uterine peroxidase activity by methoxychlor. Reprod Toxicol 8(6):477.

Cummings AM, Metcalf JM (1995). Methoxychlor regulates rat uterine estrogen-induced protein. Toxicol Appl Pharmacol 130:154.

Cummings AM (1997). Methoxychlor as a model for environmental estrogens. Crit Rev Toxicol 27(4):367-79.

Davison KL, Lamoureux CH, Feil VJ (1983). Methoxychlor metabolism in goats. 2. Metabolites in bile and movement through skin. J Agric Food Chem 31(1):164-6.

Dehal SS, Kupfer D (1994). Metabolism of the proestrogenic pesticide methoxychlor by hepatic P450 monooxygenases in rats and humans. Dual pathways involving novel ortho ring hydroxylation by CYP2B. Drug Metab Dispos 22(6):937-46.

Deichmann WB, Keplinger M, Salla F, Glass E (1967). Synergism among oral carcinogens IV. The simultaneous feeding of four tumorigens to rats. Toxicol Appl Pharmacol 11:88-103.

DeRosa C, Richter P, Pohl H, Jones DE (1998). Environmental exposures that affect the endocrine system: public health implications. J Toxicol Environ Health, Part B, 1:3-26.

DPH (2009). Maximum contaminant levels and regulatory dates for drinking water, U.S. EPA vs California. November, 2009. Division of Drinking Water and Environmental Management, California Department of Public Health, Sacramento, CA. Accessed at: http://www.cdph.ca.gov/certlic/drinkingwater/Documents/MCLreview/MCLs-DLRs-PHGs.xls.

DPR (2007) Annual pesticide use report, 2007. California Department of Pesticide Regulation, Cal/EPA. Accessed at: www.cdpr.ca.gov.

DPR (2009). Residue Monitoring Program, Residues in Fresh Produce – 2007 and 2008. California Department of Pesticide Regulation, Cal/EPA, Sacramento, CA. Accessed at: http://www.cdpr.ca.gov/docs/enforce/residue/rsmonmnu.htm.

du Pont (1951). EI du Pont de Nemours and Company, Inc., Study MRID No. 00029282, as cited by U.S. EPA (IRIS) Methoxychlor, 1998.

du Pont (1966). EI du Pont de Nemours and Company, Inc., Study MRID No. 00108732, 00113276, as cited by U.S. EPA (IRIS) Methoxychlor, 1998 (possibly same as Haskell Laboratories, 1966).

du Pont (1976). EI du Pont de Nemours and Company, Inc., Study MRID No. 00062704, as cited by U.S. EPA (IRIS) Methoxychlor, 1998.

Duff SJ, Hampson E (2000). A beneficial effect of estrogen on working memory in postmenopausal women taking hormone replacement therapy. Horm Behav 38:262-276.

Dunkel VC, Pienta RJ, Sivak A, Traul KA (1981). Comparative neoplastic transformation responses of BALB/3T3 cells, Syrian hamster embryo cells and Rauscher murine leukemia virus-infected Fischer 344 rat embryo cells to chemical carcinogens. J Natl Cancer Inst 67:1303-15.

Dwivedi C, Tabbert J (1994). Effects of methoxychlor on skin tumor development. Toxicol Lett 74(3):235-40.

Eroschenko VP (1991). Ultrastructure of vagina and uterus in young mice after methoxychlor exposure. Reprod Toxicol 5:427-35.

Eroschenko VP, Abuel-Atta AA, Grober MS (1995). Neonatal exposures to technical methoxychlor alters ovaries in adult mice. Reprod Toxicol 9(4):379-87.

Eroschenko VP, Swartz WJ *et al.* (1997). Decreased superovulation in adult mice following neonatal exposures to technical methoxychlor. Reprod Toxicol11:807-14.

Faber KA, Hughes CL (1993). Dose-response characteristics of neonatal exposure to genistein on pituitary responsiveness to gonadotropin releasing hormone and volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) in postpubertal castrated female rats. Reprod Toxicol 7:35-39.

Fei X, Chung H, Taylor HS (2005). Methoxychlor disrupts uterine Hoxa 10 gene expression. Endocrinol 146:3445-51.

Gaido KW, Leonard LS, Lovell S, Gould JC, Babai D, Portier CJ, McDonnell DP (1997). Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. Toxicol Appl Pharmacol 143(1):205-12.

Gaido KW, Leonard LS, Maness SC *et al.* (1999). Differential interaction of the methoxychlor metabolite 2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane with estrogen receptors α and ß. Endocrinol 140:5746-5753.

Gaido KW, Maness SC, McDonnell DP, Dehal SS, Kupfer D, Safe S (2000). Interaction of methoxychlor and related compounds with estrogen receptor alpha and beta, and androgen receptor: structure-activity studies. Mol Pharmacol 2000 Oct;58(4):852-8.

Gioiosa L, Fissore E, Ghiradelli G, Parmigiani S, Palanza P (2007). Developmental exposure to low-dose estrogenic endocrine disruptors alters sex differences in exploration and emotional responses in mice. Horm Behav 52:307-16.

Goldman JM, Cooper RL, Rehnberg GL, Hein JF, McElroy WK, Gray LE, Jr. (1986). Effects of low subchronic doses of methoxychlor on the rat hypothalamic-pituitary reproductive axis. Toxicol Appl Pharmacol 86:474-83.

Golovleva LA, Polyakova AB, Pertsova RN, Finkelshtein ZI (1984). The fate of methoxychlor in soils and transformation by soil microorganisms. J Environ Sci Health B 19:523-38.

Golub MS, Germann SL, Hogrefe CE (2004a). Endocrine disruption and cognitive function in adolescent female rhesus monkeys. Neurotoxicol Teratol 26:799-809.

Golub MS, Hogrefe CE, Germann SL *et al.* (2003). Effects of exogenous estrogenic agents on pubertal growth and reproductive system maturation in female rhesus monkeys. Toxicol Sci 74:103-113.

Golub MS, Hogrefe CE, Germann SL, Jerome CP (2004b). Endocrine disruption in adolescence: immunologic, hematologic and bone effects in monkeys. Toxicol Sci 82:598-607.

Gray LE Jr, Ostby J, Ferrell J, Rehnberg G, Linder R, Cooper R, Goldman J, Slott V, Laskey J (1989). A dose-response analysis of methoxychlor-induced alterations of reproductive development and function in the rat. Fund Appl Toxicol 12:92-108.

Gray LE Jr, Ostby JS, Ferrell JM, Sigmon ER, Goldman JM (1988). Methoxychlor induces estrogen-like alterations of behavior and the reproductive tract in the female rat

and hamster: Effects on sex behavior, running wheel activity, and uterine morphology. Toxicol Appl Pharmacol 96:525-40.

Gunderson E (1988). Chemical contaminants monitoring: FDA total diet study, April 1982-April 1984, dietary intakes of pesticides, selected elements, and other chemicals. J Assoc Off Anal Chem 71:1200-09.

Gupta R, Schuh R, Fiskum G *et al.* (2006). Methoxychlor causes mitochondrial dysfunction and oxidative damage in the mouse ovary. Toxicol Appl Pharmacol 216:436-45.

Haag HB, Finnegan JK, Larson PS, Reise W, Dreyfuss MI (1950). Comparative chronic toxicity for warm-blooded animals of 2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane (DDT) and 2,2-bis-(p-methoxyphenyl)-1,1,1-trichloroethane (DMDT, methoxychlor). Arch Int Pharmacodyn 83:491-504.

Hall DL, Payne LA, Putman JM *et al.* (1997). Effect of methoxychlor on implantation and embryo development in the mouse. Reprod Toxicol 11:703-8.

Harell M, Shea JJ, Emmett JR (1978). Bilateral sudden deafness following combined insecticide poisoning. Laryngoscope 88:1348-51.

Harris SJ, Cecil HC, Bitman J (1974). Effect of several dietary levels of technical methoxychlor on reproduction in rats. J Agric Food Chem 22:969-73.

Haskell Laboratories (1966). Three-generation study on rats with 2,2-bis(p-methoxyphenyl)-1,1,1-trichlorethane (methoxychlor). Haskell Laboratory Report No. 102-66. Unpublished study cited in ATSDR, 1994 (probably same study as du Pont, 1966).

Heindel JJ, Chapin RE, Gulati DK, George JD, Price CJ *et al*. (1994). Assessment of the reproductive and developmental toxicity of pesticide/fertilizer mixtures based on confirmed pesticide contamination in California and Iowa groundwater. Fund Appl Toxicol 22:605-21.

Hodge HC, Maynard EA, Blanchet HJ (1952). Chronic oral toxicity tests of methoxychlor (2,2-di-(p-methoxyphenyl)-1,1,1-trichloroethane) in rats and dogs. J Pharmacol Exp Ther 104:60-6.

Hodge, HC, Maynard EA, Thomas JF, Blanchet HJ *et al.* (1950). Short-term oral toxicity tests of methoxychlor (2,2,di-p-methoxyphenyl)-1,1,1-trichloroethane) in rats and dogs. J Pharmacol Exp Ther 99:140.

Hoff RM, Muir DC, Grift NP (1992). Annual cycle of polychlorinated biphenyls and organohalogen pesticides in air in Southern Ontario. 1. Air concentration data. Environ. Sci Technol 26:266-75.

Howard PH, ed. (1991). Handbook of environmental fate and exposure data for organic chemicals. Chelsea, MI: Lewis Publishers, Inc. pp. 502-4.

IARC (1979). IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Vol. 20: Methoxychlor. International Agency for Research on Cancer, World Health Organization, Lyon, France.

IARC (1987). IARC monographs on the evaluation of carcinogenic risk of chemicals to humans. Suppl. 7. International Agency for Research on Cancer, World Health Organization, Lyon, France.

Ito A, Fujimoto N, Okamoto T (1995). Estrogen and carcinogenesis. J Toxicol Pathol 8:285-9.

Ito N, Hasegawa R, Imaida K, Kurata Y, Hagiwara A, Shirai T (1995). Effect of ingestion of 20 pesticides in combination at acceptable daily intake levels on rat liver carcinogenesis. Fd Chem Toxic 33(2):159-63.

Ivey MC, Ivie GW, Coppock CE, Clark KJ (1983). Methoxychlor residues in milk of cattle treated with Marlate 50 insecticide as a dermal spray. J Dairy Sci 66(4):943-50.

Judy BM, Nagel SC, Thayer KA, vom Saal FS, Welshons WV (1999). Low-dose bioactivity of xenoestrogens in animals: fetal exposure to low doses of methoxychlor and other xenoestrogens increases adult prostate size in mice. Toxicol Ind Health 15:12-25.

Kapoor IP, Metcalf RL, Nystrom RF *et al*. (1970). Comparative metabolism of methoxychlor, methiochlor and DDT in mouse, insects, and in a model ecosystem. J Agric Food Chem 18:1145-52.

Keenan PA, Ezzat WH, Ginsburg K, Moore GJ (2001). Prefrontal cortex at the site of estrogen's effect on cognition. Psychoneuroendocrinol 26:577-90.

Kincaid Enterprises (1986). Rabbit teratology study with methoxychlor, technical grade. Nitro, WV. MRID No. 0015992. Unpublished study cited in U.S. EPA, 1987c, ATSDR, 1994, and U.S. EPA, 2007b.

Kupfer D, Bulger WH (1979). A novel *in vitro* method for demonstrating proestrogens. Metabolism of methoxychlor and o,p'DDT by liver microsomes in the presence of uteri and effects on intracellular distribution of estrogen receptors. Life Sci 25:975-83.

Kupfer D, Bulger WH, Theoharides AD (1990). Metabolism of methoxychlor by hepatic P-450 monooxygenases in rat and human. 1. Characterization of a novel catechol metabolite. Chem Res Toxicol 3(1):8-16.

Kupfer D, Bulger WH (1987). Metabolic activation of pesticides with proestrogenic activity. Fed Proc 46(5):1864-9.

Kurata M, Hirose K, Umeda M (1982). Inhibition of metabolic cooperation in Chinese hamster cells by organochlorine pesticides. Gann 73(2):217-21.

Latchoumycandane C, Chitra KC, Mathur PP (2002). The effect of methoxychlor on the epididymal antioxidant system of adult rats. Reprod Toxicol 16:161-172.

Levy JR, Faber KR, Ayyash L, Hughes CL (1995). The effects of prenatal exposure to the phytoestrogen genistein on sexual differentiation in rats. Proc Soc Exp Biol Med 208:60-66.

Li HC, Dehal SS, Kupfer D (1995). Induction of the hepatic CYP2B and CYP3A enzymes by the proestrogenic pesticide methoxychlor and by DDT in the rat. Effects on methoxychlor metabolism. J Biochem Toxicol 10(1):50-61.

Mann DR, Plant TM (2002). Leptin and pubertal development. Semin Reprod Med 20:93-102.

Maness SC, McDonnell DP, Gaido KW (1998). Inhibition of androgen receptor-dependent transcriptional activity by DDT isomers and methoxychlor in HepG2 human hepatoma cells. Toxicol Appl Pharmacol 151(1):135-42.

Martinez EM, Swartz WJ (1991). Effects of methoxychlor on the reproductive system of the adult female mouse. 2. Gross and histologic observations. Reprod Toxicol 5:139-47.

Martinez EM, Swartz WJ (1992). Effects of methoxychlor on the reproductive system of the adult female mouse. 2. Ultrastructural observations. Reprod Toxicol 6:93-8.

Masutomi N, Shibutani M, Takagi H *et al.* (2003). Impact of dietary exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period on the development of the rat endocrine/reproductive systems later in life. Toxicol 192:149-70.

McLachlan JA, Newbold RR, Li S, Negishi M (1998). Are estrogens carcinogenic during development of the testes? APMIS 106(1):240-2.

McLachlan JA (1997). Synergistic effect of environmental estrogens: report withdrawn. Science 277:462-3.

Metcalf JL, Laws SC, Cummings AM (1996). Methoxychlor mimics the action of 17ß-estradiol on induction of uterine epidermal growth factor receptors in immature female rats. Reprod Toxicol 10(5):393-9.

Metcalf JL, Laws SC, Cummings AM (1995). Methoxychlor mimics the action of 17ß estradiol on induction of uterine epidermal growth factor receptors in immature female rats. Biol Reprod 52 (Supp 1):97.

Mitchell AD, Rudd CJ, Caspary WJ (1988). Evaluation of L5178Y mouse lymphoma cell mutagenesis assay: Intralaboratory results for sixty-three coded chemicals tested at SRI International. Environ Mol Mutagen 12:37-101.

Muir DC, Yarechewski AL (1984). Degradation of methoxychlor in sediments under various redox conditions. J Environ Sci Health 3:271-95.

Murono E, Derk R, Akgul Y (2006). *In vivo* exposure of young adult male rats to methoxychlor reduces serum testosterone levels and *ex vivo* Leydig cell testosterone formation and cholesterol side-chain cleavage activity. Reprod Toxicol 21:148-53.

Murono EP, Derk RC (2004). The effects of the reported active metabolite of methoxychlor, 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane, on testosterone formation by cultured Leydig cells from young adult rats. Reprod Toxicol 19:135-46.

Myhr BC, Caspary WJ (1988). Evaluation of L5178Y mouse lymphoma cell mutagenesis assay: Intralaboratory results for sixty-three coded chemicals tested at Litton Bionetics, Inc. Environ Mol Mutagen 12:103-94.

NCI. (1978). Bioassay of methoxychlor for possible carcinogenicity. National Cancer Institute, Dept. of Health, Education and Welfare, Washington, DC. Tech Rep Series No. 35.

Nelson KG, Takahashi T, Bossert NL *et al.* (1991). Epidermal growth factor replaces estrogen in the stimulation of female genital-tract growth and differentiation. Proc Natl Acad Sci 88:21-5.

Newbold RR (1999). Hormonal mechanisms in female reproductive tract toxicity. In: Endocrine and Hormonal Toxicology. Harvey PW, Rush KC, Cockburn A (Eds.), Wiley, Chichester, pp. 406-17.

Oberly TJ, Michaelis KC, Rexroat MA, Bewsey BJ, Garriott ML (1993). A comparison of the CHO/HGPRT+ and the L5178Y/TK+/- mutation assays using suspension treatment and soft agar cloning: results for 10 chemicals. Cell Biol Toxicol 9(3):243-57.

Odom J, Lefevre PA, Tittensor S, Paton D, Routledge EJ, Beresford NA, Sumpter JP, Ashby J (1997). The rodent uterotrophic assay: critical protocol features, studies with nonyl phenols, and comparison with a yeast estrogenicity assay. Regul Toxicol Pharmacol 25(2):176-88.

OEHHA (2005). Development of health criteria for school site risk assessment pursuant to Health and Safety Code Section 901(g): Child-specific reference doses for school site risk assessment – cadmium, chlordane, heptachlor, heptachlor epoxide, methoxychlor, and nickel. Final Report, December, 2005. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA. Accessed at: http://www.oehha.ca.gov/public_info/public/kids/pdf/FinalSchoolReport121205.pdf.

Okazaki K, Okazaki S, Nishimura S, Nakamura H *et al.* (2001). A repeated 28-day oral dose toxicity study of methoxychlor in rats, based on the "enhanced OECD test guideline 407" for screening endocrine-disrupting chemicals. Arch Toxicol 75: 513-521.

Ousterhout JM, Struck RF, Nelson JA (1979). Estrogenic properties of methoxychlor metabolites. Fed Proc Fed Am Soc Exp Biol 38(3):537.

Palanza P, Morellini F, Parmigiani S, vom Saal FS (2002). Ethological methods to study the effects of maternal exposure to estrogenic endocrine disruptors: a study with methoxychlor. Neurotoxicol Teratol 24:55-69.

PAN (2009). Pesticide Action Network. Accessed at: www.pesticideinfo.org.

Probst GS, McMahon RE, Hill LE, Thompson CZ, Epp JK, Neal SB (1981). Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: A comparison with bacterial mutagenicity using 218 compounds. Environ Mut 3:11-32.

Ramamoorthy K, Wang F, Chen I-C, Safe S, Norris JD *et al.* (1997). Potency of combined estrogenic pesticides. Science 275:405.

Reuber MD (1979a). Carcinomas of the liver in Osborne-Mendel rats ingesting methoxychlor. Life Sci 24:1367-72.

Reuber MD (1979b). Interstitial cell carcinomas of the testis in balb/C male mice ingesting methoxychlor. Cancer Res Clin Oncol 93:173-9.

Reuber MD (1980). Carcinogenicity and toxicity of methoxychlor. Environ Health Perspect 36:205-19.

Safe S (1998). Interactions between hormones and chemicals in breast cancer. Ann Rev Pharmacol Toxicol 38:121-58.

Sax NI (1987). Methoxychlor. In: Dangerous Properties of Industrial Materials. New York: Van Nostrand Reinhold. Suppl. Sept/Oct 1987, pp. 79-87.

Shain SA, Shaeffer JC, Boesel RW (1977). The effect of chronic ingestion of selected pesticides upon rat ventral prostate homeostasis. Toxicol Appl Pharmacol 40:115-30.

Shelby MD, Newbold RR, Tully DB, Chae K, Davis VL (1996). Assessing environmental chemicals for estrogenicity using a combination of in vitro and in vivo assays. Environ Health Perspect 104(12):1296-300.

Shin JH, Moon H, Kang IH *et al.* (2007). Calbindin-d9k mRNA expression in the rat uterus following exposure to methoxychlor: a comparison of oral and subcutaneous exposure. J Reprod Dev 53:179-88.

Simmon VF (1979). *In vitro* microbiological mutagenicity and unscheduled DNA synthesis studies of 18 pesticides. EPA 600/1-79-041.

Skaare JU, Berge G, Odegaard S, Grave K (1982). Excretion of methoxychlor in cow milk following dermal application. Acta Vet Scand 23(1):16-23.

Staub C, Hardy V, Chapin R, Harris M, Johnson L (2002). The hidden effect of estrogenic/antiandrogenic methoxychlor on spermatogenesis. Toxicol Appl Pharmacol 180:129-35.

Stein AA (1968). Comparative methoxychlor toxicity in dogs, swine, rats, monkeys, and man. Med Surg 37:540-1.

Stocco DM, Wells J, Clark BJ (1993). The effects of hydrogen peroxide on steroidogenesis in mouse Leydig tumor cells. Endocrinol 133:2827-32.

Stresser DM, Dehal SS, Kupfer D (1996). Ring hydroxylation of [o-^{3}H]methoxychlor as a probe for liver microsomal CYP2B activity: potential for *in vivo* CYP2B assay. Anal Biochem 233(1):100-7.

Suzuki M, Lee HC, Chiba S *et al.* (2004). Effects of methoxychlor exposure during perinatal period on reproductive function after maturation in rats. J Reprod Dev 50:455-61.

Swartz WJ, Corkern M (1992). Effects of methoxychlor treatment of pregnant mice on female offspring of the treated and subsequent pregnancies. Reprod Toxicol 6(5):431-7.

Swartz WJ, Eroschenko VP (1998). Neonatal exposure to technical methoxychlor alters pregnancy outcome in female mice. Reprod Toxicol 12(6):565-73.

Symonds D, Tomic D, Miller K *et al.* (2005). Methoxychlor induces proliferation of the mouse ovarian surface epithelium. Toxicol Sci 83:355-62.

Symonds D, Miller K, Tomic D *et al.* (2006). Effect of methoxychlor and estradiol on cytochrome p450 enzymes in the mouse ovarian surface epithelium. Toxicol Sci 89:510-4.

Symonds D, Merchenthaler I, Flaws J (2008). Methoxychlor and estradiol induce oxidative stress DNA damage in the mouse ovarian surface epithelium. Toxicol Sci 105(1):182-7.

Takagi H, Mitsumori K, Onodera H *et al*. (2002). Improvement of a two-stage carcinogenesis model to detect modifying effects of endocrine disrupting chemicals on thyroid carcinogenesis in rats. Cancer Lett 178:1-9.

Tegeris AS, Earl FL, Smalley HE *et al*. (1966). Methoxychlor toxicity: Comparative studies in the dog and the swine. Arch Environ Health 13:776-87.

Terasawa E, Fernandez D (2001). Neurobiological mechanisms of the onset of puberty in primates. Endocrin Rev 22:111-51.

Thompson TS, Vorster SJ (2000). Attempted suicide by ingestion of methoxychlor. J Anal Toxicol 24:377-80.

Tiemann U (2008). *In vivo* and *in vitro* effects of the organochlorine pesticides DDT, TCPM, methoxychlor, and lindane on the female reproductive tract of mammals: a review. Reprod Toxicol 25:316-26.

Toppari J, Christiansen P, Giwercman A, Grandjean P, Guillette LJ Jr *et al*. (1996). Male reproductive health and environmental xenoestrogens. Environ Health Perspect 104 (Suppl 4):741-803.

Traul KA, Takayama K, Kachevsky V, Hink RJ, Wolf JS (1981). Rapid *in vitro* assay for carcinogenicity of chemical substances in mammalian cells utilizing an attachment-independent endpoint. 2. Assay validation. J Appl Toxicol 1:190-5.

Troiano J, Weaver D, Marade J, Spurlock F, Pepple M, Nordmark C, Bartkowiak D (2001). Summary of well water sampling in California to detect pesticide residues resulting from nonpoint-source applications. J Environ Qual 30:448-59.

Tullner WW (1961). Uterotrophic action of the insecticide methoxychlor. Science 133:647-8.

U.S. EPA (1976). Quality criteria for water, July 1976. U.S. Environmental Protection Agency, Washington, D.C. PB-263943.

U.S. EPA (1987a). Drinking water criteria document for methoxychlor. Office of Health and Environmental Assessment, U.S. Environmental Protection Agency, Washington, D.C. PB89-192215.

U.S. EPA (1987b). Methoxychlor health advisory. Office of Drinking Water, U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA (1987c). Review of teratology study in rabbits with methoxychlor (Kincaid Enterprises, 1986). Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency, Washington, D.C. MRID 005881.

U.S. EPA (1988). Pesticide Fact Sheet: Methoxychlor. Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency, Washington, D.C. EPA 540/FS-89-014.

U.S. EPA (1990a). Non-occupational pesticide exposure study (NOPES). Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. EPA 600/3-90/003.

U.S. EPA (1990b). National Pesticide Survey. Summary results of EPA's national survey of pesticides in drinking water wells. Office of Water, U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA (1996). Guidelines for Reproductive Toxicity Risk Assessment. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. Federal Register 61(212):56273-322, EPA 630/R-96/009a.

U.S. EPA (1997). Special report on environmental endocrine disruption: an effects assessment and analysis. Prepared by a technical panel for the U.S. EPA Risk Assessment Forum. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. EPA 630/R-96/012.

U.S. EPA (2004a). Methoxychlor Reregistration Eligibility Decision (RED). U.S. Environmental Protection Agency, Washington, D.C. Publ. No. EPA 738-R-04-010. Accessed at: http://www.epa.gov/oppsrrd1/REDs/methoxychlor_red.htm.

U.S. EPA (2004b). Estimated per capita water ingestion and body weight in the United States – an update. EPA/822/R-00/001. October, 2004. Accessed at: http://www.epa.gov/waterscience/drinking/percapita/2004.pdf.

U.S. EPA (2006). National Recommended Water Quality Criteria. Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency. Accessed at: http://www.epa.gov/waterscience/criteria/wqcriteria.html.

U.S. EPA (2009). Consumer Factsheet on Methoxychlor. Office of Ground Water and Drinking Water, U.S. Environmental Protection Agency, Washington, D.C. Accessed at: http://www.epa.gov/safewater/pdfs/factsheets/soc/methoxyc.pdf.

U.S. EPA (2010). Methoxychlor, oral RfD last revised 8/91, cancer assessment updated 10/90. Integrated Risk Information System (IRIS), U.S. Environmental Protection Agency, Washington, D.C. Accessed at: www.epa.gov/iris.

U.S. FDA (1996). Pesticide program, residue monitoring 1995. U.S. Food and Drug Administration, Washington, D.C.

Uzumcu M, Kuhn PE, Marano JE *et al*. (2006). Early postnatal methoxychlor exposure inhibits folliculogenesis and stimulates anti-Mullerian hormone production in the rat ovary. J Endocrin 191:549-58.

Uzumcu M, Zachow R (2007). Developmental exposure to environmental endocrine disruptors: consequences within the ovary and on female reproductive function. Reprod Toxicol 23:337-52.

Vaithinathan S, Saradha B, Mathur P (2008). Transient inhibitory effect of methoxychlor on testicular steroidogenesis in rat: an in vivo study. Arch Toxicol 82:833-9.

Vaithinathan S, Saradha B, Mathur PP (2009). Methoxychlor-induced alteration in the levels of HSP70 and clusterin is accompanied with oxidative stress in adult rat testis. J Biochem Mol Toxicol 23(1):29-35.

vom Saal FS, Quadagno DM, Even MD, Keisler LW *et al*. (1990). Paradoxical effects of maternal stress on fetal steroids and postnatal reproductive traits in female mice from different intrauterine positions. Biol Reprod 43:751-61.

vom Saal FS, Nagel SC, Palanza P, Boechler M, Parmigiani S, Welshons WV (1995). Estrogenic pesticides: binding relative to estradiol in MCF-7 cells and effects of exposure during fetal life on subsequent territorial behaviour in male mice. Toxicol Lett 77:343-50.

von Schoultz B, Carlstrom k, Collste L *et al*. (1989). Estrogen therapy and liver function – metabolic effects of oral and parenteral administration. Prostate 14:389-95.

Walters LM, Rourke AW, Eroschenko VP (1993). Purified methoxychlor stimulates the reproductive tract in immature female mice. Reprod Toxicol 7:599-606.

Wang XJ, Bartolucci-Page E, Fenton S, You L (2006). Altered mammary gland development in male rats exposed to genistein and methoxychlor. Toxicol Sci 91(1):93-103.

Wania F, Mackay D (1996). Tracking the distribution of persistent organic pollutants. Environ Sci Technol 30:390-6.

Ward M., Colt J, Metayer C *et al.* (2009). Residential exposure to polychlorinated biphenyls and organochlorine pesticides and risk of childhood leukemia. Environ Health Perspect 117(6):1007-13.

Waters MD, Sandhu SS, Simmon VF *et al*. (1982). Study of pesticide genotoxicity. Basic Life Sci 21:275-326. As cited in U.S. EPA, 1987.

Welch HE, Muir DC, Billeck BN *et al*. (1991). Brown snow: A long-range transport event in the Canadian arctic. Environ Sci Technol 25:280-6.

Welshons WV, Thayer KA, Judy BM, Taylor JA, Curran EM, vom Saal FS (2003). Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. Environ Health Perspect 111(8):994-1006.

Wenda-Rozewicka L (1983). Morphometric studies of male gonads from mice receiving insecticides (Metox-30, Sadofos-30 and Foschlor-50). Folia Biol 32:23-34.

Wester RC, Maibach HI, Bucks DAW, Sedik L, Melendres J, Liao C, DiZio S (1990). Percutaneous absorption of ^{14}C-DDT and ^{14}C-benzo[a]pyrene from soil. Fund Appl Toxicol 15:510-6.

WHO (2004). Methoxychlor in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. World Health Organization, Geneva. WHO/SDE/WSH/03.04/105. Accessed at: http://www.who.int/water_sanitation_health/dwq/chemicals/methoxychlor.pdf.

You L, Casanova M, Bartolucci EJ *et al*. (2002). Combined effects of dietary phytoestrogen and synthetic endocrine-active compound on reproductive development in Sprague-Dawley rats: genistein and methoxychlor. Toxicol Sci 66:91-104.

Zhou LX, Dehal SS, Kupfer D, Morrell S, McKenzie BA, Eccleston ED Jr., Holtzman JL (1995). Cytochrome P450 catalyzed covalent binding of methoxychlor to rat hepatic, microsomal iodothyronine 5'-monodeiodinase, type I: does exposure to methoxychlor disrupt thyroid hormone metabolism? Arch Biochem Biophys 322(2):390-4.

Ziem G (1982). Aplastic anaemia after methoxychlor exposure. Lancet, December 11, 1349.