ADDENDUM TO THE TOXICOLOGICAL PROFILE FOR METHOXYCHLOR

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CONTENTS

ADDENDUM FOR Methoxychlor
Supplement to the 2002 Toxicological Profile for Methoxychlor

Background Statement

This addendum for Methoxychlor supplements the Toxicological Profile for Methoxychlor that was released in 2002.

Toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). CERCLA mandates that the Administrator of ATSDR prepare toxicological profiles on substances on the Priority List and that the profiles be revised "no less often than once every three years". CERCLA further states that the Administrator will "establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" [Title 42, Chapter 103, Subchapter I, § 9604 (i)(1)(B)].

The purpose of this addendum is to provide, to the public, other federal, state, and local agencies a non-peer reviewed supplement of the scientific data that was published in the open peer-reviewed literature since the release of the profile in 2002.

Chapter numbers in this addendum coincide with the toxicological profile for Methoxychlor (2002). This document should be used in conjunction with the profile. It does not replace it.

2. RELEVANCE TO PUBLIC HEALTH

2.3 MINIMAL RISK LEVELS (MRLs)

No changes in MRLs from the *Toxicological Profile* for Methoxychlor (2002).

3. HEALTH EFFECTS

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

3.2.2 Oral Exposure

3.2.2.3 Immunological and Lymphoreticular Effects

White et al. (2005) evaluated the immunotoxicity of methoxychlor in F0 (dams) and F1 generations of Sprague-Dawley rats exposed to an isoflavone-free diet containing methoxychlor at concentrations of 10, 100, and 1000 ppm. In dams, exposure to methoxychlor from gestation day 7 to postpartum day 51 (65 days total exposure) produced a significant increase in natural killer cell activity (1000 ppm) and in the percentages of T cells (1000 ppm), helper T cells (1000 ppm), and macrophages (100 and 1000 ppm). In contrast, a decrease in the numbers of splenocytes and B cells was observed at the 100 and 1000 ppm concentrations. In F1 males, exposure to methoxychlor gestationally, lactationally, and through feed from postnatal day 22–64 (78 days total exposure) produced an increase in the spleen IgM antibody-forming cell response to sheep red blood cells (100 and 1000 ppm) and the activity of NK cells (1000 ppm). However, there was a decrease in the terminal body weight (1000 ppm), spleen weight (1000 ppm), thymus weight (100 and 1000 ppm), and the numbers of splenocytes (1000 ppm), B cells (100 and 1000 ppm), cytotoxic T cells (1000 ppm), and NK cells (100 and 1000 ppm). In F1 females, exposure to methoxychlor produced a decrease in the terminal body weight (1000 ppm) and the percentages of cytotoxic T cells (10, 100, and 1000 ppm).

These results demonstrate that developmental and adult dietary exposure to methoxychlor modulates immune responses in Sprague-Dawley rats. Immunological changes were more pronounced in the F1 generation male rats that were exposed during gestation and postpartum than in the F0 and F1 generation females. Increases in antibody-forming cell response and NK cell activity and altered spleen cell subpopulation numbers were observed in the F1 generation male rats, without similar changes to the F1 generation females.

Golub et al. (2004) examined the effect of treatment with estrogenic agents [methoxychlor (MXC), 25 and 50 mg/kg/day; diethylstilbestrol (DES), 0.5 mg/kg/day] on immune, hematologic, and bone mass parameters given in the peripubertal period to female rhesus monkeys. DES affected several hematological and clinical chemistry parameters including hematocrit, hemoglobin, serum albumin, liver transaminases, and lipids. Circulating lymphocytes, particularly B cells, were depressed by DES, and a maturational shift in a memory T-cell population was altered. In addition, bone mass and length, as measured after a 9-month recovery period, were significantly reduced in the DES group, and bone mass tended to be reduced in the femur of the MXC50 group relative to controls.

3.2.2.6 Developmental Effects

Gioiosa et al. (2007) investigated the effects of maternal exposure to estrogenic endrocrine disruptors—at concentrations within the range of human exposure and not patently teratogenic—on behavioral responses of male and female house mice (Mus musculus domesticus) before and after puberty. Pregnant dams were trained to spontaneously drink daily doses of corn oil with or without the estrogenic plastic derivative bisphenol A (BPA 10 µg/kg) or MXC (20 µg/kg) from gestation day 11 to postpartum day 8. Male and female offspring were examined at different ages to ascertain several components of explorative and emotional behaviors in 3 experimental paradigms: (1) a novelty test before puberty and, as adults, (2) a free-exploratory open-field test and (3) the elevated plus maze test. Control mice exhibited a number of sex-related differences in behavioral responses in both age groups and in all experimental paradigms.

In contrast, perinatal exposure to BPA or MXC decreased or eliminated such sex-related differences.

Takeuchi et al. (2002) examined the effect of MXC on the thymus in rat pups that were delivered from dams receiving MXC at a dietary concentration of 0 or 1500 ppm from gestation day through lactation. Pups of both sexes were euthanized on postnatal days (PNDs) 7, 14, and 21. Histologically, the thymus showed marked depletion of cortical lymphocytes on PND 7 and also an increase in lymphophagocytosis in the cortical area on PNDs 14 and 21. Morphometrical analysis disclosed that both cortex and medulla of the thymus from treated pups were reduced in size, but the reduction was more evident in the cortex. A significant increase in transferase-mediated dUTP nick end labeling-positive cells was detected in the cortex area, corresponding to the presence of lymphophagocytosis. Flow cytometric analysis revealed a significant decrease in the double positive (CD3(int)CD4(+)CD8(+)) immature cells on PND 21. These results have suggested that MXC may impair maturation of thymic lymphocytes in rat pups, with a resulting enhancement of apoptosis leading to thymic atrophy during the postnatal period.

3.3 GENOTOXICITY

Goldman et al. (2004) focused on whether the observed differences in tissue growth between uterus and pituitary in response to MXC administration were paralleled by a corresponding disparity in the expression of those growth factors (members of the vascular endothelial growth factor [VEGF] and angiopoietin families and their receptors) that are involved in the angiogenic cascade. Ovariectomized adult Sprague-Dawley female rats were administered MXC (0–200 mg/kg, oral) for 1 or 3 weeks. Immunohistochemical staining of uteri and pituitaries was performed under strictly controlled conditions for VEGF and its receptor VEGFR2, Angiopoietin-1 (Ang1), and angiopoietin-2 and their tyrosine kinase receptor Tie2, and platelet endothelial adhesion factor (as an index of vascularity). The results showed uterine MXC-induced increases in the expression of VEGFR2 and Ang1, changes consistent with a normal proliferative response to estrogenic stimulation. For VEGF, staining tended to be most pronounced in the stromal region, although there did not appear to be a progressive increase with dose.

VEGFR2 expression showed significant dose-related trends in luminal and glandular epithelia by 1 week. Similar effects at 1 week were evident for Ang1 in glandular epithelium. In the anterior pituitary, a dose-related increase in VEGF was present for the 1- and 3-week treatments, and the number of pituitary vessels per unit area was also increased after 3 weeks. The effects indicate that even though the insecticide has not been found to cause an augmentation in pituitary growth, a dose-related rise in the expression of at least one principal angiogenic factor is present that may be associated with an increase in vascular density.

Steffens et al. (2007) examined the effect of the MXC on skeletal muscle development, using C2C12 cell culture. Myoblast cultures were exposed to various concentrations of MXC at various times during the process of myoblast fusion into myotubes. MXC exposure decreased myotube formation and induced cytoplasmic vacuolization. Because cytoplasmic vacuoles can be characteristic of cell death, apoptosis assays and trypan blue exclusion assays were performed. No differences were found in either the frequency of apoptosis or the frequency of cell death for cultures exposed to MXC compared to untreated cultures. Collectively, these results indicate that MXC exposure decreases myotube formation without causing cell death. In contrast, when cell proliferation was assessed, untreated cultures had a myoblast proliferation rate 50% greater than cultures exposed to MXC. Steffen et al. (2007) concluded that MXC decreases myotube formation at least in part by slowing myoblast proliferation, an effect that could impact skeletal muscle development.

3.4 TOXICOKINETICS

3.4.4 Elimination and Excretion

Hazai et al. (2004) studied the estrogenic activity of methoxychlor in mammals in vivo. Methoxychlor undergoes oxidative metabolism by cytochromes P450, yielding 1,1,1-trichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane (mono-OH-M) and 1,1,1-trichloro-2,2-bis(4-hydroxyphenyl)ethane (bis-OH-M) as main metabolites. Since, humans may be exposed to these estrogenic metabolites, which are potential substrates of

UDP-glucuronosyltransferases (UGTs), their glucuronide conjugation was investigated with human liver preparations and individual UGTs. Incubation of both mono-OH-M and bis-OH-M with human liver microsomes formed monoglucuronides. The structures of the glucuronides were identified by liquid chromatography/tandem mass spectrometry. Examination of cDNA-expressed recombinant human hepatic UGTs revealed that several catalyze glucuronidation of both compounds. Among the cDNA-expressed UGT1A enzymes, UGT1A9 seemed to be the main catalyst of formation of mono-OH-M-glucuronide, whereas UGT1A3 seemed to be the most active in bis-OH-M-glucuronide formation. Furthermore, the chiral selectivity of mono-OH-M glucuronidation was examined. The results of the incubation of single enantiomers generally agreed with the chiral analyses of mono-OH-M derived from the glucuronidase digestion of the glucuronides of the racemic mono-OH-M. There was a relatively slight but consistent enantioselective preference of individual UGT1A1, UGT1A3, UGT1A9, and UGT2B15 enzymes for glucuronidation of the S- over the R-mono-OH-M, whereas in human liver microsomes, differences were observed among donors in generating the respective R/S-mono-OH-M ratio. Since it was previously shown that human liver microsomes demethylate methoxychlor mainly into S-mono-OH-M, the observation that UGT1A isoforms preferentially glucuronidate the S-mono-OH-M suggests a suitable mechanism for eliminating this major enantiomer.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Lafuente et al. (2008) evaluated the effects of MXC on the hypothalamic-pituitary-testicular axis in adult male rats. This global objective comprised three major aims: (1) to analyze the possible differential MXC effects in norepinephrine and serotonin concentration in serotoninergic metabolism in anterior, mediobasal, and posterior hypothalamus and median eminence; (2) to evaluate effects induced by MXC exposure on gonadotropins and testosterone; and 3) to elucidate whether the regulatory interactions in the hypothalamic-pituitary-testicular axis are modified by this pesticide. Animals were administered subcutaneously 25mg/kg/day of MXC for 1 month. MXC increased

norepinephrine and serotonin content in anterior hypothalamus ($P \leq 0.05$), but decreased serotonin concentration in posterior hypothalamus ($P \leq 0.05$). MXC diminished serotonin turnover in anterior hypothalamus ($P \leq 0.01$) and decreased plasma LH ($P <$ or $= 0.001$) and testosterone ($P \leq 0.05$) levels, but those of FSH remained unmodified. It might be concluded that MXC exposure (1) could exert differential effects in norepinephrine and serotonin concentration in serotoninergic metabolism in anterior, mediobasal, and posterior hypothalamus and median eminence, with the anterior hypothalamus the most sensitive region to the pesticide; (2) could inhibit LH and testosterone secretion without changing FSH; (3) might involve four potential pathways in MXC effects on testosterone secretion (changing LH secretion; modifying serotonin and norepinephrine at the hypothalamic level; alterating the direct neural pathway between brain and testes; and/or by a direct effect in testes).

4. CHEMICAL AND PHYSICAL INFORMATION

No updated data.

5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

No updated data.

6. POTENTIAL FOR HUMAN EXPOSURE

No updated data.

7. ANALYTICAL METHODS

No updated data.

8. REGULATIONS AND ADVISORIES

No updated data.

9. REFERENCES

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