19 March 2010

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Re: **Endocrine Disruptor Screening Program Tier 1 Screening Order Issuing Announcement (74 FR 54422); EPA–HQ–OPP–2009–0634**

Addendum to Comments submitted February 13, 2010
Chlorpyrifos CAS number 2921-88-2
Test order numbers EDSP-059101-30 through 35
Test order date: November 5, 2009

Here we present information to augment our comments on chlorpyrifos submitted on February 13, 2010.

Human Toxicity:

Human studies were conducted to quantify a NOEL for ***erythrocyte AChE inhibition***. The study was a *"double blind, randomized, placebo controlled, rising dose design conducted in two phases separated by 14 days"* (Kisicki et al 1999). Phase 1 had three dose levels (0, 0.5, and 1mg/kg) and Phase 2 had 2 doses (0, 2mg/kg). The study authors concluded that a single dose of 1mg/kg is the NOAEL for erythrocyte AChE inhibition and 2mg/kg is the threshold value (Kisicki et al 1999). In an older study, incarcerated males were dosed (voluntarily) to determine the effect threshold for adult men with prolonged daily brief exposure. Study authors determined the threshold level to be at or below 0.01mg/kg/day (Coulston et al 1972).

Reproductive Toxicity (rodent, rabbit, dog animal models):

In a ***multigenerational reproductive study***, rats were exposed to chlorpyrifos to determine effects on reproductive capacity and fertility. Male and female Sprague-Dawley rats were treated with chlorpyrifos 10 weeks before and during mating (F0). Females were further exposed during pregnancy and lactation (until weaning of the F1 generation on PND 21) and F1 rats raised the F2 generation until weaning. Daily doses of chlorpyrifos were 0, 0.1, 1, and 5 mg /kg. There was no indication of increased prenatal mortality up to 5 mg/kg . There was ***no decrease in fertility*** within the F0/F1 or the F1/F2 generation, up to a daily dose of 5 mg/kg, which, according to previous studies, represents the threshold for inducing effects on both neonatal development (body weight and survival) and maternal toxicity. Animals mated with normal frequency and exhibited ***normal pregnancies, offspring and lactation*** (Breslin et al., 1996).

In an inhalation study, ***no effects on testicular weight and histology*** were reported in Fischer 344 rats treated with doses of chlorpyrifos at 0.3 mg/m3, 6 h/day, 5 days/week, for 13 weeks. These levels were insufficient to inhibit erythrocyte or plasma cholinesterase activity via inhalation exposure (Corley et al., 1989).

In a ***developmental and reproductive study***, rabbits were dosed with chlorpyrifos and maternal NOAEL and developmental NOAEL were reported as 81 mg/kg. There was ***no adverse effect on offspring development*** in this study (Rubin et al. 1987b).

Older data also confirm a lack of reproductive effect of chlorpyrifos. ***No effect on testes weight or reproductive organ histology*** was seen in Sherman rats or beagle dogs chronically treated with up to 3 mg/kg chlorpyrifos for 1–2 years (McCollister et al., 1974).

Chlorpyrifos Metabolites:

Chlorpyrifos is metabolized very quickly and is well absorbed from the intestine and from the lung in both humans and experimental animals. Chlorpyrifos degradation pathway yields four known metabolites: TCPy, DEP, DETP, and ***chlorpyrifos-oxon***.

Following oral exposure, quantifiable levels of chlorpyrifos metabolites can be found in the urine of rats at 24 h and at 48 hours DEP and TCPy are still detected. The major urinary metabolites were TCPy (62%) and DETP (40%); DEP was a rather minor metabolite (4%). Chlorpyrifos could not be detected in the urine (Timchalk et al., 2007). In a case of accidental poisoning in humans, 16 metabolites were detected in the patient's urine but neither the parent compound chlorpyrifos nor chlorpyrifos-oxon was detected (Bicker et al 2005a).

> "*Several of these putative active metabolites are likely to be formed in the liver, the brain, and the developing fetus. Some of these metabolites have either been shown to interact with biological components or may be anticipated to do so. Chlorpyrifos-oxon is considered to be the sole active metabolite responsible for the* ***inhibition of AChE***" (Eaton et al. 2008).

Manifestation of Acute Cholinesterase Inhibition:

The primary targets for organophosphate pesticides (OP) like chlorpyrifos are cholinesterases (BuChE and AChE), whose physiological role is that of hydrolyzing acetylcholine, a major neurotransmitter in the central and peripheral nervous systems. Inhibition of cholinesterases can cause an accumulation of acetylcholine in cholinergic synapses which can cause over-stimulation of muscarinic and nicotinic receptors. A "cholinergic syndrome" ensues, causing increased sweating, salivation, bronchial secretion, diarrhea, tremors, muscular twitching, and various central nervous system effects. "*When death occurs, this is believed to be due to respiratory failure caused by inhibition of respiratory centers in the brainstem, bronchoconstriction and increased bronchial secretion, and flaccid paralysis of respiratory muscles"* (Eaton 2008).

Summary:

An enormous library of toxicological data exists for chlorpyrifos, dating back to 1965. The weight of the evidence shows a very clear pathway of metabolism and chemical degradation. Reproductive data is also plentiful and shows ***no reproductive effects on fertility, sex organs or development*** in animal experiments at high exposures. Human data is also available and shows AChE inhibition at levels well below those causing overt toxicity. Inhibition of AChE as the major mode of action for chlorpyrifos is very clear in human and other animal studies, as well as *in vitro*. Since AChE inhibition occurs at exposures well below no effect levels seen for reproductive effects, whether or not chlorpyrifos can also modulate estrogen or androgen activity is irrelevant to its regulation. If it is determined that the estrogen or androgen receptor-modulating capacity of chlorpyrifos is required for some as yet undefined reason, this could easily be established using *in vitro* methods, those in the Tier 1 battery and/or in ToxCast.

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