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Chlorpyrifos: Weight of evidence evaluation of potential interaction with the estrogen, androgen, or thyroid pathways

Daland R. Juberg [a], Sean C. Gehen [a], Katie K. Coady [b], Matthew J. LeBaron [b], Vince J. Kramer [a], Haitian Lu [a], M. Sue Marty [b,*]

[a] Dow AgroSciences LLC, 9330 Zionsville, Road, Indianapolis, IN 46268, USA
[b] Toxicology & Environmental Research and Consulting, The Dow Chemical Company, 1803 Building, Midland, MI 48674, USA

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ABSTRACT

Chlorpyrifos was selected for EPA's Endocrine Disruptor Screening Program (EDSP) based on widespread use and potential for human and environmental exposures. The purpose of the program is to screen chemicals for their potential to interact with the estrogen, androgen, or thyroid pathways. A battery of 11 assays was completed for chlorpyrifos in accordance with test guidelines developed for EDSP Tier 1 screening. To determine potential endocrine activity, a weight-of-evidence (WoE) evaluation was completed for chlorpyrifos, which included the integration of EDSP assay results with data from regulatory guideline studies and the published literature. This WoE approach was based on the OECD conceptual framework for testing and assessment of potential endocrine-disrupting chemicals and consisted of a systematic evaluation of data, progressing from simple to complex across multiple levels of biological organization. The conclusion of the WoE evaluation is that chlorpyrifos demonstrates no potential to interact with the estrogen, androgen, or thyroid pathways at doses below the dose levels that inhibit cholinesterase. Therefore, regulatory exposure limits for chlorpyrifos, which are based on cholinesterase inhibition, are sufficient to protect against potential endocrine alterations. Based on the results of this WoE evaluation, there is no scientific justification for pursuing additional endocrine testing for chlorpyrifos.

1. Introduction

The term "endocrine disruptor" or "endocrine disrupting chemical" was put forward in recent years by members of the scientific community based on the hypothesis that certain substances may have intrinsic properties allowing interaction with and potential "disruption" of the normal function of the endocrine system. Subsequently, various efforts have been undertaken to ascribe an unambiguous definition to the designation "endocrine disruptor". One widely accepted definition agreed at the 1996 Weybridge Conference (now termed the Weybridge-definition) describes an endocrine disruptor as "an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function".

To address concerns that environmental chemicals could act as endocrine disruptors and cause adverse health effects, provisions were included in the 1996 Food Quality Protection Act as well as amendments to Safe Drinking Water Act, both of which called for the US EPA to screen chemicals, including pesticides, for potential endocrine disrupting properties. In order to comply with these provisions, EPA initiated efforts in the mid-to-late 1990s to develop a screening program designed to identify potential endocrine-disrupting chemicals. Termed the Endocrine Disruptor Screening Program (EDSP), this EPA effort was developed as a two-tiered screening and testing program. The stated purpose of Tier 1 screening, which includes 11 assays, is to "identify substances with the potential to interact with the estrogen, androgen, or thyroid hormone systems". Tier 2 is designed to determine: (1) "whether a substance may cause endocrine-mediated effects through or involving estrogen, androgen, or thyroid hormone systems"; (2) "the consequences to the organism of the activities observed in Tier 1"; and (3) "the relationship between doses of an endocrine-active substance administered in the test and the effects observed" (US EPA, 2005). Five tests have been proposed for Tier 2 EDSP.

In 2007, EPA released a draft initial list of 73 chemicals for screening which included primarily pesticide active ingredients with a small number of HPV/pesticide "inert" chemicals (US EPA, 2007a). One chemical included on the initial EDSP screening list was chlorpyrifos (*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl)ester phosphorothioic acid), a widely-used organophosphate pesticide product. As with other compounds on priority list 1 for endocrine screening, chlorpyrifos' selection was not due to effects, but was based on criteria for potential for exposure through diet (food

* Corresponding author. Fax: +1 989 638 9863.
E-mail addresses: msmarty@dow.com, mmarty@dow.com (M.S. Marty).

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and water), residential use, and occupational contact with treated surfaces (US EPA, 2002; 2007a). The exposure potential was based on EPA review of existing databases and those chemicals with potential for exposure through multiple pathways were considered to have a higher priority for inclusion on EDSP list 1. Of note, in June 2000, chlorpyrifos was voluntarily withdrawn from residential uses in the US, thereby reducing residential exposures (US EPA, 2011a).

Chlorpyrifos is a pesticide product widely used in the US and other countries to manage insect pests on many agricultural crops including tree fruits and nuts, vines, vegetables, corn and soybeans. It is a member of the organophosphate class of insecticides and was first introduced for use in the US in 1965. Since that time, it has become one of the most widely studied pesticides from both human health and environmental perspectives.

In 2009, a testing consortium was developed to respond to the Tier 1 EDSP test orders for chlorpyrifos. In accordance with the EPA EDSP, the consortium opted to submit existing data (i.e., other scientifically relevant information (OSRI)) to fulfill a subset of the EDSP assay requirements. However, based on some methodological differences and a desire to have a complete EDSP Tier 1 data set, it was subsequently agreed to conduct all 11 Tier 1 assays. The Tier 1 battery for chlorpyrifos was completed and submitted to EPA in 2011 in accordance with the test order 24-month response period.

To determine the potential for endocrine activity, a weight-of-evidence (WoE) evaluation was completed which included the integration of EDSP assay results with existing data from regulatory guideline studies and the published literature. In order to produce the WoE evaluation of the chlorpyrifos EDSP data-set, several existing WoE frameworks and methodologies were first evaluated (Borgert et al., 2011; Boobis et al., 2008; Rhomberg, 2010; Weed, 2005) for their potential utility in conducting this evaluation. While some common features from these approaches were adopted (e.g., reliance on a hypothesis-based approach, the need to appropriately organize and integrate information, the need for critical evaluation of data relevance and reliability), it was determined that the overall clarity and reliability of the evaluation would be benefited by utilizing an endocrine-specific approach to the degree possible. While the Borgert et al. approach is specific to the issue of endocrine disruption and EDSP, it was determined at the time of the chlorpyrifos evaluation to not be sufficiently developed for the purposes of producing a clear and straightforward WoE document. Thus, based on the currently availability of methodologies, it was determined that a qualitative endocrine-specific WoE evaluation approach would be utilized.

One common feature of all the approaches was the need to first organize all relevant information in a meaningful manner. To accomplish this requirement, it was decided to base the WoE evaluation on the approach adopted under the OECD conceptual framework for testing and assessment of potential endocrine-disrupting chemicals. The conceptual framework represents the consensus agreement of the OECD endocrine task force and thus can be considered as a well vetted and established approach. One purpose of the framework is to facilitate the organization of data across multiple levels of biological complexity in a straightforward manner (OECD, 2010). This feature was important for the EDSP data-set given the different test systems used (*in vitro* vs *in vivo*) and the need to put this information into the appropriate context. Since the framework was developed specifically for addressing the topic of endocrine disruption, the test systems and approaches utilized for EDSP are included specifically in the framework and thus can easily be incorporated into the appropriate level for the WoE evaluation. Specifically, the framework consists of five levels of information including: (1) non-test information (e.g., physical–chemical properties); (2) *in vitro* assay results; (3) *in vivo* assays that inform on endocrine pathways; (4) *in vivo* apical assays that evaluate specific endocrine endpoints; and (5) *in vivo* assays that provide comprehensive data on potential adverse effects over multiple life stages of an organism. Thus, in general data obtained in higher-tier test systems would carry more weight than those from lower tiers. This approach is similar to that recommended by the European Centre for Toxicology and Ecotoxicology of Chemicals (ECETOC) in which higher-tier (i.e., *in vivo* apical) studies are more important than lower- tier (i.e., *in vitro* or single endpoint *in vivo*) studies in determining whether a chemical has endocrine disrupting properties (Bars et al., 2011).

In addition to the newly developed data under this Tier 1 screening, an extensive review was undertaken of published scientific literature and multiple studies conducted under good laboratory practices (GLP) for pesticide registration purposes, both of which span a host of endpoints that inform on endocrine disruption potential. Where relevant and reliable data was identified, this information was also incorporated into the appropriate level of the framework and ultimately included in the WoE evaluation.

The purpose of this manuscript is to summarize the results of the Tier 1 EDSP results for chlorpyrifos and provide a WoE-based conclusion, based on the approach used in this particular assessment, on the potential for chlorpyrifos to interact with the estrogen, androgen or thyroid pathways. A WoE approach is important, because the Tier 1 battery was designed to include highly sensitive assays in order to minimize "false negative" results, which increases the likelihood of "false positive" findings (EDSTAC, 1998). Consequently, undue weight should not be placed on any single result. Isolated positive findings need to be interpreted carefully with the overall pattern of effects across the battery being the key finding, allowing firm conclusions on potential endocrine activity.

2. Methods

2.1. Tier 1 EDSP assays

EDSP Tier 1 assays were conducted in accordance with the identified test guidelines and are briefly described in Table 1.

2.2. Weight of evidence (WoE)

While there are many variations on conducting a WoE evaluation (e.g., Borgert et al., 2011; Boobis et al., 2008; Rhomberg, 2010; Weed, 2005), there are several concepts in common between the various approaches including: (1) gathering and organizing all relevant information; (2) determining quality and reliability of data; (3) integrating data from different sources in a manner that facilitates evaluation and comparison of data from different sources; (4) evaluating data for factors such as consistency, coherence, adequacy, plausiblity, etc.; and (5) employing a weighting or hypothesis-based evaluation system to draw conclusions on the existing dataset (Borgert et al., 2011; Boobis et al., 2008; Rhomberg, 2010; Weed, 2005).

In order to produce a transparent and scientifically valid endocrine WoE evaluation for chlorpyrifos, existing approaches were utilized to determine the reliability of information and ascribe appropriate weight to the evidence. The general approach adhered to was as follows: (1) gather all available information including EDSP data, pesticide registration studies, and literature search results relevant to determining the potential for chlorpyrifos to interact with the estrogen, androgen or thyroid pathways; (2) Determine reliability of literature using **Tox**icological data **R**eliability Assessment **Tool** (ToxRTool) (US EPA, 2011b); (3) Organize relevant and reliable evidence according the 5-level OECD conceptual framework for the Testing and Assessment of Endocrine Disrupting