Regulatory Toxicology and Pharmacology 64 (2012) 263–266

Contents lists available at SciVerse ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Commentary

Implications of estimates of residential organophosphate exposure from dialkylphosphates (DAPs) and their relevance to risk

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ARTICLE INFO

Article history:
Received 14 May 2012
Available online 23 August 2012

Keywords:
Organophosphorus insecticide
Dialkyl phosphate
DAPs
Epidemiology
Pesticide residue

ABSTRACT

Recent epidemiological studies have claimed to associate a variety of toxicological effects of organophosphorus insecticides (OPs) and residential OP exposure based on the dialkyl phosphates (DAPs; metabolic and environmental breakdown products of OPs) levels in the urine of pregnant females. A key premise in those epidemiology studies was that the level of urinary DAPs was directly related to the level of parent OP exposure. Specific chemical biomarkers and DAPs representing absorbed dose of OPs are invaluable to reconstruct human exposures in prospective occupational studies and even in non-occupational studies when exposure to a specific OP can be described. However, measurement of those detoxification products in urine without specific knowledge of insecticide exposure is insufficient to establish OP insecticide exposure. DAPs have high oral bioavailability and are ubiquitously present in produce at concentrations several-fold greater than parent OPs. Studies relying on DAPs as an indicator of OP exposure that lack credible information on proximate OP exposure are simply measuring DAP exposure and misattributing OP exposure.

1. Commentary

The purpose of this commentary is to focus concern on studies that claim to demonstrate a relationship between a variety of epidemiologically associated toxicological effects of OPs and residential OP exposure based on the dialkyl phosphates (DAPs; metabolic and environmental breakdown products of OPs) levels in the urine of pregnant females. A key premise in those epidemiology studies was that the level of urinary DAPs was directly related to the level of parent OP exposure, i.e., the DAPs are being used as surrogates for cumulative exposure from several different OPs either methyl and/or ethyl. However, although worker cohort studies have concurrently measured DAP urinary excretion and cholinesterase (Kraus et al., 1977; Drevenkar et al., 1991; Aprea et al., 1994; McCurdy et al., 1994; Krieger and Dinoff, 2001; Cocker et al., 2002; Wessel et al., 2003; Johnstone et al., 2007), they do not show a consistent correlation with acetylcholinesterase (AChE) inhibition). To our knowledge there are no human studies of residential exposure using DAPs with evidence of concurrent AChE inhibition, i.e., there is no demonstrated relationship of DAPs and change in AChE except in occupational settings where there was clear and unambiguous exposure to pesticidal levels of OPs.

Urinary DAPs have been used as biomarkers for OP exposure in workers since the 1970s (Shafik et al., 1976). However, unlike most residential exposure studies, data supporting studies in the workplace for the mixer/loader/applicator include knowledge of specific OPs used and most urinary DAPs are due to OP compounds, not their breakdown products. Workers reentering treated fields several days after OP applications may be exposed to substantial amounts of DAPs as the parent OPs naturally dissipate, but at this time we have no knowledge of dermal DAP bioavailability. More recently, urinary DAP levels have been used as indicators of cumulative OP exposure in the general population (Barr et al., 2011a,b; Payne-Sturges et al., 2009). Urinary DAP's have also been used as markers for OP exposures in pregnant women in a variety of residential epidemiological studies (Bouchard et al., 2010, 2011; Bradman et al., 2003, 2005; Castorina et al., 2003; Engel et al., 2007; Eskenazi et al., 2004; Harari et al., 2010; Lizardi et al., 2008; Marks et al., 2010; McKone et al., 2007; Rauch et al., 2012; Rohlman et al., 2005; and Young et al., 2005). The primary source of exposure to the subjects of these studies was likely low level residues (OP and DAP) in the food (Barr et al., 2011a,b). Although as McKone et al. (2007) have noted "...more published research is needed to elucidate the metabolism and excretion of preformed DAPs in the

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http://dx.doi.org/10.1016/j.yrtph.2012.08.012

environment". There is general acknowledgment that DAPs are available from food (Timchalk et al., 2007; Forsberg et al., 2011; Barr et al., 2011a,b; Chen et al., 2012b).

The possibility that OP exposure estimated from DAP excretion may primarily reflect direct intake of DAP residues tends to be ignored or dismissed in epidemiology studies. For example, Bouchard et al. (2010) state "In any case, misclassification of exposure on the basis of measurements of urinary DAP levels should be nondifferential and should bias effect estimates toward the null." We note that measuring DAP levels as a surrogate for measuring OP levels is not the same thing as measuring OP levels imprecisely. That is DAP levels are measured precisely and may accurately reflect exposure to foods treated at some time in the past with OP's, but not exposure to OP's themselves. To the extent that exposure to these hypothetical foods is associated with health effects, either positive or negative, the effects could be wrongly attributed to OP exposure.

One recent article that does acknowledge exposure issues is by Engel et al. (2011) in which they stated "Thus, for subjects for whom the primary source of pesticide exposure is fresh fruit and vegetable consumption, use of urinary metabolite concentrations as an indication of parent compound exposure may result in significant misclassification of exposure." However, there is no reason to believe that exposure to DAPs in the diet would be limited to just fresh fruit and vegetables. For example, although we know of no actual measurements, dry grains, dry fruit, dry vegetables or any canned or processed commodity that had at some point been treated with an OP will likely contain DAPs that exceed the levels of parent OP in the fresh commodities on a molar basis given the stability and persistence of DAPs in fresh commodities (Chen et al., 2012a; Zhang et al., 2008). This is because dehydrating produce concentrates salts, for example DAPs, and heating during the canning process will increase the hydrolysis of OPs to DAPs. Despite acknowledgement that "urinary DAP measurements reflect exposure to non-toxic preformed metabolites as well as the parent (toxic) OP pesticides, particularly for dietary exposures," Rauch et al. (2012) conclude that the effects they observed on birthweight and gestational age were due to parent OP exposure, although parent OP exposures were never actually measured.

Based upon a wealth of research on mammalian metabolism it is clear that for most cases, the urinary DAP data are inadequate measures of residential exposure for quantitative risk assessment. For the general population, an absorbed OP dose cannot be distinguished in the urine from an absorbed dose of the detoxified breakdown products. The basis for concern with the use of urinary DAPs as a quantitative measure of residential OP exposure is that on average DAPs are present at concentrations 6-fold greater than OPs on or in treated produce (Zhang et al., 2008; Chen et al., 2012a). The DAPs derive from both environmental decay and metabolism of OPs (Duggan et al., 2003). For example, a study indicated that under field conditions, DAPs present in treated strawberries were much more persistent than the parent OP (Li, 2009). Thus, a large source of DAPs in biomonitored urine is almost certainly from direct intake of DAPs in foodstuffs (Duggan et al., 2003; Lu et al., 2005). Additionally, there is likely substantial DAPs exposure resulting from the 1/3rd of all produce treated with OPs that have non-detectable levels of OPs during multiresidue analysis, i.e., the DAP residues tend to be conserved as the OPs dissipate. For example, as malathion dissipates following a single application in strawberries, the ratio of DAPs/parent increased from 4 to 14 over a 20 day interval (Chen et al., 2012a).

A key factor in analyzing biomonitoring data is knowledge of the pharmacokinetics of metabolites being used as biomarkers of exposure. Consequently, a key question that needs to be answered is to what extent, if any, does oral absorption of DAPs occur? The primary ethyl homologue DAPs found in urine (diethyl phosphate and diethyl thiophosphate) are well-absorbed and excreted in urine ($\geqslant$50%) following oral dosing in rats (Timchalk et al., 2007). Research with dimethyl phosphates shows similar results (Chen et al., 2012b; Forsberg et al., 2011). Thus, DAPs have demonstrated high oral bioavailability. Perhaps more importantly, once absorbed the DAPs are either excreted unchanged, or for DAPs containing sulfur are partially oxidized to their oxygen analogues prior to excretion (Chen et al., 2012b).

EPA is at a crossroads in regulating the OP insecticides. An updated Registration Eligibility Decision (RED) for chlorpyrifos (one of the most widely used OPs in agriculture) was released in early July, 2011 and EPA has independently committed to producing a revised cumulative OP assessment perhaps this year. The chlorpyrifos RED cited 3 epidemiologic study cohorts that EPA suggested provided support for effects well below the LOAELs for cholinesterase inhibition (EPA, 2011). The OP cumulative risk assessment document will no doubt revisit the issue of the best method to estimate exposure to more than 20 different OPs currently registered in the US. As virtually all home uses of OPs were eliminated in 2001, the primary source of non-occupational human exposure to OPs remaining is dietary. Ambient air levels of OPs even in intensive agricultural areas can account for only a small fraction of a theoretical residential total OP dose (McKone et al., 2007).

While acknowledging the overestimation bias that DAPs bring to residential exposure, it would be interesting to compare them to EPA's modeled estimates of cumulative OP exposure to get some idea of relative magnitude. However, it is very difficult to determine what EPA's (2001) cumulative OP dosage was. This is because EPA presents the OP cumulative exposure data as toxicological methamidophos dose equivalents. Methamidophos is not only one of the most potent AChE inhibitors, but it also has one of the lowest molecular weights of all the OPs, and thus EPA's cumulative OP dose estimate requires a correction for both factors. However, any estimate of OP dosage should also take into account that the most potent OPs are increasingly the least used. Simply summing the average dietary estimate (50th percentile) alone for all of the OPs (0.00117 mg/kg; Duggan et al., 2003) suggests there is a severe bias in EPA's 95th percentile methamidophos normalized OP cumulative exposure estimate (0.0001 mg/kg) (EPA, 2001). Urinary biomonitoring data of DAPs collected during the National Health and Nutrition Examination Survey (NHANES) and reported by CDC (Barr et al., 2011a,b) provides an alternative method for estimating cumulative OP exposure. The cumulative absorbed dose of OPs based on the assumption that DAPs in the urine represent only ingested parent compounds was estimated to be approximately 0.0003 mg/kg-day (arithmetic average, Cochran, 2002), or 0.00034 mg/kg-day (geometric mean, Duggan et al., 2003) nationwide. These biomonitoring-based cumulative OP exposure estimates integrated all sources including home uses of OPs. Home use of OPs was still permitted at the time the NHANES urine samples were taken in 1999–2000 (CDC, 2009). The most recent publicly available NHANES survey from 2003–2004 (CDC, 2009), provided urinary DAP data that indicated that the average cumulative OP exposure without in home uses was 0.18 μg/kg-day. The above biomonitoring exposure estimates are based on the assumption that all of the DAPs in the urine came from the parent compounds. Because urinary DAPs probably overestimate residential OP exposure by a factor of >6 based on relative molar ratios of DAPs to OPs in food (Zhang et al., 2008), a biomonitoring dose estimated from DAPs could provide an extreme upper bound for comparison with EPA's mass-based cumulative OP assessment.

Thus, it appears that estimates of residential OP exposures based on urinary DAP levels will tend to greatly overestimate true exposure, and it is unclear whether modeled exposure estimates are even comparable. The question then arises as to how much this