Preformed Biomarkers in Produce Inflate Human Organophosphate Exposure Assessments

We read the recent report by Curl et al. (2003) concerning organophosphate (OP) pesticide exposures with considerable interest. It seems to have escaped their notice that OP pesticides yield the same dialkylphosphate (DAP) products in urine whether they are human metabolites or formed in treated fruits and vegetables and consumed in the diet. Because the same metabolites arise from foods, the conclusions of Curl et al. regarding eating conventional and organic diets are not justified.

Curl et al. (2003) measured urine levels of DAPs of children 2–5 years of age and claimed different exposures based on whether the children consumed organic or conventional diets. Parents kept a food diary during the 2 days before the day of urine sampling. Eighteen of the children ate nearly all organic produce and juice; 21 others consumed conventional produce and juice. Not surprisingly, the children that consumed the conventional diets had more nontoxic DAPs in their urine than did those on an organic diet. The most prominent DAP metabolites were dimethylthiophosphate (DMTP) and diethylthiophosphate (DETP). The median total dimethyl metabolite concentration was about six times higher for children with conventional diets than for children with organic diets. The investigators erred by attributing the urinary DAPs to ingested OP pesticides in foods.

Additionally, Curl et al. (2003) transformed the dimethyl metabolites to low, benign levels of oxydemeton-methyl (2.2 µg/kg/day), azinphosmethyl (2.8 µg/kg/day), phosmet (2.8 µg/kg/day), and malathion (2.3 µg/kg/day). Curl et al. (2003) concluded,

> Consumption of organic produce appears to provide a relatively simple means for parents to reduce their children's exposure to organophosphate pesticides.

Others have been quick to agree. Richard Wiles, Environmental Working Group (Lyman F. Unpublished data), stated: "… this is the first study to document the differences in exposures to pesticides offered by an organic versus a conventional diet…." Charles Benbrook (Unpublished data) has declared the work, "the most compelling new study to appear on pesticide dietary risks in a long time…." *Science News* also subscribed to the same notion (Haber 2003). The conclusions of Curl et al. (2003) and those of their enthusiastic readers are not justified by available data.

Hydrolytic scission of the most electronegative-leaving group of an OP pesticide generates the respective DAPs. In plants and animals, this is an important degradation pathway of organophosphates. The metabolites include dimethyl phosphate, DMTP, dimethydithiophosphate, diethyphosphate, DETP, and diethyldithiophosphate. These chemicals are collectively termed "DAPs." The pK_a values for these DAPs range from 1.25 to 1.62 (Eto 1979). DAPs are ionized in animals at physiologic pH (7.4). Ionization contributes to very high water solubility. In the stomach at pH 1–2, approximately one-half of each DAP would be un-ionized, making them more readily absorbed from the gastrointestinal tract. Thus, DAPs from the diet or drinking water can be absorbed and excreted in the urine. Any meat and milk residues from the diet would be far below limits of detection.

Our preliminary food analyses (unpublished data) and the literature of OP metabolism in plants developed over nearly 50 years [e.g. Casida (1961) and references therein] support our observation that urinary DAPs at low levels represent both human OP metabolites and preformed plant OP degradation products. When ^{32}P was a relatively common radiolabel for metabolic studies, research unequivocally established the occurrence of DAPs in a variety of plants. We have found nontoxic DAPs in 12 of 12 produce samples from the channels of trade in central California. The produce was selected because each had been shown to contain an OP residue during routine monitoring by shippers and producers. All residues were below established residue tolerances. Pesticides in the pilot study included cadusafos, chlorpyrifos, diazinon, dimethoate, ethoprop, malathion, omethoate, oxydemeton-methyl, and terbufos. The mole ratios of DAP metabolites to parent OP residues ranged from 0.1 to > 130. Six of 12 samples contained more DAP residue than the parent OP. The interval between pesticide application and other agronomic factors will probably be an important determinant of the ratio of DAP to OP in produce. Consumer urine DAPs, therefore, represent both preformed plant metabolites and human metabolites resulting from detoxification of the pesticide residue. All preformed metabolites represent false positives in any attempt to directly back-calculate OP exposure of children or adults (Curl et al. 2003).

The possible contributions of the food supply to DAP in urine have been given virtually no scientific consideration. In the recent *Second National Report on Human Exposure to Environmental Chemicals* [Centers for Disease Control and Prevention (CDC) 2003], other sources of DAPs were noted. The report states that ingestion of food contaminated with organophosphorous pesticides and contact during residential application is the main source of exposure for the general population. The dietary assumption is not questioned, but excretion of DAPs represents both preformed DAPs and those resulting from trace OP residues in food. Residential application is an additional source of low-level exposure in places where OP use is still permitted. The CDC report states that DAPs may be present in the environment from degradation of OPs but continues to attribute urinary metabolites to the parent insecticide.

When preformed DAPs in the diets of the children in the University of Washington studies are considered, the pesticide exposures reported by Curl et al. (2003) are inflated to an unknown extent. Clearly the statement from Philip Landrigan (Mount Sinai School of Medicine, New York, NY) that "the sheer presence of a metabolite shows exposure to the toxic pesticides" (Curl et al. 2003) is misleading to consumers and must be adjusted to the reality that both plants and people break down OP pesticides to DAPs (Lyman F. Unpublished data).

DAP in urine is the sum of metabolites from trace OP residue in the food and preformed DAP from produce. The sources of these nontoxic DAPs will vary with individual produce, and they cannot be distinguished by urine testing. Scientific studies intended to detect extremely low, benign levels of DAP must consider all sources that contribute to human exposure.

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Pesticide Exposures and Children's Risk Tradeoffs

Evidence available thus far does not support the conclusion by Curl et al. (2003) that parents' choice of organic produce reduces children's risks. Choosing organic produce simply changes children's risks. In their article, "Organophosphate Pesticide Exposure of Urban and Suburban Preschool Children with Organic and Conventional Diets," Curl et al. (2003) offered suggestive evidence supporting the hypothesis that children who eat "organic" produce are less at risk from the potential effects of pesticide exposure because they have fewer organophosphate (OP) metabolites in their urine. While it does appear that the group of children the authors tested who ate mostly conventional produce had higher levels of urinary OP metabolites than the group who ate mostly organic produce, judgments about their relative risk cannot be supported on that basis.

Curl et al. (2003) stated that consumption of organic produce shifts children's OP exposures "from a range of uncertain risk to a range of negligible risk." Actually, consumption of organic produce shifts children from a range of almost certainly negligible risk due to potential OP exposures to a range of uncertain risk due to fungal toxins and plant stress-mediated increases in allergens (Midoro-Horiuti et al. 2001) and naturally occurring plant toxins (Beier and Nigg 1994; Wood 1979). Plants use complex chemistry to defend themselves from insects, fungi, viruses, bacteria, and larger herbivores. The need for natural chemical defenses is particularly critical for organically grown produce, which is not otherwise defended by synthetic chemicals. In fact, when plants have to devote more energy to self-defense, they have less energy to devote to nutrient content (e.g., Ojimelukwe et al. 1999).

There are admittedly few reports that directly contrast the levels of natural plant pesticides in organic and nonorganic produce. One example is organically grown parsnips, which have more than twice the levels of genotoxic furocoumarins (also present in carrots, celery, and oranges) than conventional parsnips (Mongeau et al. 1994). The concentrations of furocoumarins in both conventional and organic parsnips are three orders of magnitude higher than the concentrations of synthetic pesticides (U.S. Department of Agriculture 2000). Another example is the use of fungicides on wheat, which reduces the level of mycotoxins to about one-third that found in untreated wheat (Hicks et al. 1999). Although the relationship between crop protection and decreased natural toxicant levels is largely inferential, there is a large literature documenting the relationship between crop stress and increased levels of plant toxicants (Mattsson 2000 and references cited therein). A particularly well-documented example is the response of potatoes to stress and infection by elevating glycoalkaloid concentrations (Kuc 1973). The toxic properties of glycoalkaloids include anticholinesterase activity, nausea, diarrhea, abdominal pain, and death in humans (Friedman and McDonald 1997) and birth defects and increased fetal mortality in laboratory animals (Friedman et al. 2003; Gaffield and Keeler 1996). When produce is grown organically, it is subject to greater stress from pests than when it is grown with synthetic pesticides.

OP and other anthropogenic pesticides have been subjected to extensive toxicologic testing to meet the U.S. Environmental Protection Agency's requirements for registration. Naturally occurring chemical pesticides are not systematically tested for toxic effects. Those natural pesticides that have been tested are just as capable of producing toxicity in laboratory animals under experimental conditions as are anthropogenic pesticides. To be registered, the risks from anthropogenic pesticide products are well characterized and limited to negligible levels by law. The risks from naturally occurring chemical pesticides are seldom characterized or limited by law. A 1996 National Academy of Sciences report concluded that "… natural components of the diet may prove to be of greater concern than synthetic components …" (National Academy of Sciences/National Research Council 1996).

Most risk decisions involve tradeoffs. It is often the case that reducing one risk increases another. In Curl et al.'s example (Curl et al. 2003), reducing one fairly well-characterized risk most likely increases another fairly well-uncharacterized risk, pointing out an important problem that is receiving inadequate attention. There is a clear need to investigate and characterize the risk tradeoffs associated with the use or omission of synthetic pesticides.

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Organophosphate Exposure: Response to Krieger et al. and Charnley

Our recent study of children's dietary exposure to organophosphorus (OP) pesticides (Curl et al. 2003) has elicited two very different responses from readers. In that paper we demonstrated a 6-fold difference in median dialkylphosphate (DAP) concentrations in the urine of children who consumed primarily organically grown or conventionally grown produce. We concluded that consumption of organic rather than conventional produce would result in a reduction of OP pesticide exposure for these children. Krieger et al. respond to our study by suggesting that, since some fraction of the DAP compounds measured in urine samples could be the result of exposure to DAPs present in foods, our conclusions are "not justified by available data." However, Krieger et al. provide little evidence in their letter to support their argument that DAP concentrations measured in urine are the result of DAPs in food.

Krieger et al. mistakenly state that "it seems to have escaped notice" that breakdown