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 Richard Wiles, Environmental Working Group (Lyman F. Unpublished data), stated: "… this is the first study to document the differences in exposures to pesticides offered by an organic versus a conventional diet.…" Charles Benbrook (Unpublished data) has declared the work, "the most compelling new study to appear on pesticide dietary risks in a long time.…" *Science News* also subscribed to the same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

products of OP pesticides occur in the environment, including food. In fact, this issue has been discussed at numerous scientific meetings in recent years, and has been well documented during the investigations of methyl parathion misapplications conducted by the Centers for Disease Control and Prevention (Hryhorczuk et al. 2002; Imtiaz and Haugh 2002; Rubin et al. 2002). In regard to the DAP compounds specifically, we raised this issue several years ago in our discussion of biologically based dose estimates of OP pesticide exposure (Fenske et al. 2000). The reason that the issue has not received more prominence is that data to support this viewpoint are not available. At present the argument is largely hypothetical, and Krieger et al. provide only a modicum of new information to inform this discussion.

It is certainly true that pesticide breakdown products can be present in the environment and in food. However, the article cited by Krieger et al. to support this point (Casida 1961) makes clear that OP pesticides can be metabolized by plants to a number of compounds, including the mono-alkyl phosphates and phosphoric acid, none of which would impact DAP measurements in urine. Thus, the extent to which urinary DAPs are by-products of plant metabolism remains unclear. Krieger et al. also cite preliminary data from their own laboratory as evidence of DAPs in food. If correct, these findings would be helpful documentation of the extent to which DAP formation can occur in dietary samples, and we look forward to the publication of these data.

It is also possible, as Krieger et al. argue, that some fraction of DAPs present on food could be absorbed through the gastrointestinal tract, but to our knowledge no studies have been conducted to estimate the extent of such absorption. Finally, their argument rests on the assumption that DAPs, once absorbed, will pass through the body unchanged and appear in the urine. Krieger et al. do not even acknowledge this assumption, and no data are provided to support it. We know of only one study that has attempted to examine the fate of the DAPs in biological systems (Imaizumi et al. 1993). In this study, rats were administered high doses of several DAP compounds. Only small fractions of the administered doses were recovered in urine, suggesting that these compounds were metabolized rather than excreted unchanged. Furthermore, at least one of the DAPs (diethylthiophosphate) inhibited cholinesterase in the rat brain, raising a question as to whether the DAP compounds themselves carry some risk, in contrast to the assertion by Krieger et al. that these compounds are "nontoxic."

In summary, Krieger et al. have put forth a criticism based largely on conjecture. We cannot conclude from their evidence that the 6-fold difference we report in urinary DAP concentrations between the two groups of children in our study can be explained by DAPs in food. After all, it is well recognized that conventionally grown foods contain more pesticides than do organic foods (Baker et al. 2002). We believe that differential exposure to OP pesticides is a far more likely explanation of this observed difference. We welcome further research in this area, as it will assist all of us in our efforts to develop more accurate estimates of human exposures to hazardous chemicals.

In a second response to our study, Charnley calls attention to the presence of natural toxins in food, echoing a view set forth recently by Mattsson (2000). Charnley is critical of our statement that consumption of organic produce shifts children's OP pesticide exposures from a range of uncertain risk to a range of negligible risk because we have not accounted for risks associated with natural toxins. This criticism raises an important question regarding the proper framework for risk evaluation. More than two decades of study have shown that the context in which risk is evaluated is fundamental to how one goes about the calculation of risk (Presidential/Congressional Commission on Risk Assessment and Risk Management 1997; Slovic 1987).

We stated very clearly that the framework for our risk evaluation is based on "current U.S. Environmental Protection Agency guidelines" (Curl et al. 2003). Charnley (in her letter) and Mattsson (2000) would broaden the risk framework for pesticides to include natural plant toxins, and their argument is beguiling: Plants produce toxins as a stress response to predators; pesticides reduce predator populations and therefore stress; ergo, pesticide-treated plants have less stress and can produce more nutritious chemicals. What is wrong with this proposition, which seems to take us through the looking glass to a world where pesticides are a kind of chemical therapy for plants?

First, this argument rests on a reductionist view of sustainable agriculture in which organic farming is simply conventional farming without pesticides. In fact, sustainable agriculture promotes a host of cultivation practices that differ markedly from the practices of what is often referred to as industrial agriculture (Horrigan et al. 2002). None of the studies cited by Charnley report results from organic farming practices. It seems plausible that many of the practices that differentiate organic and industrial agriculture could be at play in the elaboration of natural plant toxins, and not simply pesticide use.

Second, analysis of the risks of natural toxins is most certainly more complex than Charnley indicates. To take but one example, she notes that glycoalkaloids in potatoes have demonstrated toxic effects in laboratory studies. However, these chemicals also appear to have beneficial health effects, such as conferring protection from viral and bacterial infections (Friedman et al. 2003), a fact that neither Charnley nor Mattsson (2000) choose to mention. Similarly, a recent study of conventional and organic crops indicated that plants grown organically produced higher levels of antioxidants, a presumed benefit to human health (Asami et al. 2003). Charnley also overlooks the fact that many plant toxins would greatly degrade the taste and palatability of fruits and vegetables at doses sufficient to produce acute toxicity in humans (Drewnowski and Gomez-Carneros 2000).

Beyond these arguments, it should be noted that natural toxins generally fall outside of current regulatory boundaries. As Charnley herself points out, natural toxins are "seldom characterized or limited by law." Her argument thus seeks to expand the scope of risk assessment beyond that considered in our analysis. However, a full accounting of the risks associated with pesticide use would need to include several other types of risk, including those faced by agricultural workers. Workers who handle pesticides or who enter pesticide-treated fields can suffer serious illnesses from overexposures to these compounds. For instance, the California Environmental Protection Agency (2002) reported five confirmed "group poisonings" due to OP pesticides in 2000, affecting a total of 151 workers. Farming with pesticides makes such events possible and raises an important environmental justice issue: Should agricultural workers be placed at greater risk so that consumers can purchase foods with reduced natural toxin content? The risk evaluation framework could be further expanded to include community pesticide exposures (e.g., spray drift, groundwater contamination) and ecologic risks.

As our society not so long ago examined pesticide risks from each source in isolation (e.g., residues on food, residential use) and evaluated chemicals with a common mechanism of action separate from one another (e.g., OP pesticides), today we compartmentalize occupational, ecologic, and consumer risks. However, a fully comprehensive risk analysis will need to integrate all of these risks to produce a fair evaluation of the use of pesticides in agriculture.

Both Krieger et al. and Charnley raise important issues. However, we believe that the difference in children's exposure levels found in our study cannot be dismissed based on the evidence they provide. We look forward to future research on pesticide biomarkers and to the development of a broader evaluative framework for the analysis of the

risks and benefits of pesticide use in agriculture.

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Corn and Corn-Derived Products: Sources of Endocrine Disruptors

Markaverich et. al (2002a, 2002b) recently reported the recovery of a mitogen from corn that disrupts sexual behavior and completely blocks estrous cyclicity in rats at 0.32 mg/kg/day. The agent is a tetrahydrofuran diol (THF-diol) that also stimulates proliferation of prostate cancer cells and estrogen-dependent and -independent breast cancer cells in micromolar concentrations. It does not appear to exert its effects through the estrogen receptor, as it does not cause uterine hypertrophy. The authors speculated that the agent interferes with normal functioning of the hypothalamic–pituitary–ovarian axis. The compound is recoverable from corn cobs, whole corn kernels, and corn tortillas.

Evidence of estrogenic activity in corn oil was reported more than 40 years ago (Booth et al. 1960). This study concluded that dietary administration of oils obtained from corn, peanuts, olives, soybeans, coconuts, and rice bran increased uterine weight in mice. A 1986 study was unable to reproduce these results with corn, safflower, sunflower, or soya-bean oil (Bieber 1986). In this study, however, the control diet contained 75% corn starch and 0.5% corn oil. Test oils were substituted for corn starch at 5% or 20% of the diet.

In a study in mice, Thigpen et al. (1987) reported stimulation of uterine growth in mice by dextrose, sucrose, corn starch, corn oil, and soybean oil. Using diethylstilbestrol (DES) as a positive control, investigators determined that the relative potency of the test compounds was DES 4 ppb < sucrose < soybean oil < corn starch < dextrose < corn oil < DES 6 ppb. These results suggest that the conclusions of the 1986 study of vegetable oils (Bieber 1986) may have been influenced by the use of corn starch as a "negative" control. Other studies have also reported estrogenic activity in cane and beet molasses (Feldman et al. 1995; Miller et al. 1986).

Many plants contain estrogenic isoflavones, but they have not been specifically identified in corn that is not genetically modified. Yet, the studies cited above show that estrogenic and other endocrine-disrupting compounds are present in corn and corn-derived products, as well as other sweeteners in the human diet, though mechanistic understanding of effects is incomplete.

Some authors suggest that the n-6 fatty acid content of corn oil plays an important role in its developmental impacts. Hilakivi-Clarke et al. (1997) reported that pregnant rodents given a diet with 45% versus 15–20% of calories coming from corn oil gave birth to female offspring with earlier onset of puberty, altered mammary gland development, and increased mammary cancer risk after exposure to a carcinogen. In humans, the influence of maternal diet on breast cancer risk among daughters is of increasing concern (Hilakivi-Clarke et al. 1999).

The potential health impacts of estrogenic and other endocrine-disrupting substances present in corn, corn-derived products, and sweeteners deserve attention. Many food products contain high fructose corn syrup. Corn starch, corn oil, and corn cob bedding are used in laboratory animal studies and may be influencing results in undetected ways. Corn oil, often used as a negative control and as a vehicle for administering test substances, may have biological effects that influence outcomes and data interpretation. It is important for researchers to take this into account. Many questions remain unanswered, including the identity of the substances with hormonal activity, their origin, whether they are present in all corn hybrids, and the impact of refinement processes. The widespread use of corn oil, high fructose corn syrup, and other sweeteners in human diets lends a sense of urgency to further investigation.

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