



Could Pesticide Toxicology Studies be More Relevant to Occupational Risk Assessment?

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Pesticide toxicology study design has evolved from concern for oral exposure via food residues. The emphasis on the oral route does not generally apply to workers that are exposed primarily via the dermal route either handling pesticides or re-entering treated fields. As a result numerous assumptions about how oral toxicology results relate to dermal exposure must be made when conducting worker risk assessments. These assumptions introduce a high degree of uncertainty. Alternative toxicology study designs are suggested to reduce uncertainty when assessing risk. Because the dermal route is so important to characterizing occupational risk, methods to improve the accuracy of dermal absorption estimates are suggested, including the use of human subjects to study dermal absorption. Additional suggestions include tailoring dermal, oral and inhalation kinetic study designs to reflect worker exposure dosages. Suggestions are made to routinely conduct a single dose toxicity study patterned after the neurotoxicity study design to distinguish single dose effects and NOAELs from those resulting from multiple doses. Finally, interspecies pharmacokinetics studies are proposed to determine which toxicology study regimen of dosing best reflects intermittent worker exposure. © 2001 Published by Elsevier Science Ltd on behalf of British Occupational Hygiene Society

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INTRODUCTION

Regulation of pesticides has evolved in the United States primarily from concern about dietary exposure. The 1906 Pure Food and Drug Act prohibited unsafe substances in food, and a later statute, the Insecticide Act of 1910, established product-labeling provisions. The Federal Insecticide, Fungicide, and Rodenticide act of 1947 (FIFRA) required registration of pesticide products with the US Department of Agriculture prior to domestic or foreign sales. The Federal Food, Drug, and Cosmetics Act which evolved from the 1906 statute, was expanded in 1954 by the Miller amendment. The amendment established pesticide tolerances in or on agricultural commodities based primarily upon good agricultural practices. Soon thereafter, the

Delaney Clause of 1958 prohibited use of any carcinogenic food additive in processed foods. Broad and more unified regulatory authority developed with the 1970 formation of the US Environmental Protection Agency and an additional 1972 FIFRA amendment which required manufacturers to demonstrate that use of the product 'would not cause adverse effects on human health or the environment.'

Emphasis on promoting food safety has been reflected both in laws regulating pesticides (Food Quality Protection Act) and the regulatory agencies that have been historically responsible for enforcing those laws (Food and Drug Administration, 1958–1972 and US Department of Agriculture 1920–1958). As the required pesticide toxicology studies under FIFRA evolved from, and still closely mirror Food and Drug Administration requirements, they emphasize continuous exposure through the oral route. Thus, they were not designed with worker risk assessment in mind. Nonetheless, regulatory agencies must attempt to relate the toxicological dose response in

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those studies to the exposure of workers. However, worker exposure to pesticides tends to be intermittent in nature, and mainly via the dermal route (Krieger and Ross, 1993). Because of regulatory focus on dietary exposure, risk assessments for workers are driven by toxicology data generated in laboratory animals with a disparate route, frequency and duration of exposure.

In addition to characterizing 'traditional' dose-response in toxicology studies, the frequency and duration of exposures (in addition to magnitude) required to elicit pathology should be considered (Ecobichon, 1992). The route-specific exposure level, frequency and duration (collectively referred to as a multi-dimensional 'exposure metric') required to elicit a given toxicological effect should be considered when deciding what exposure scenario to compare it to for purposes of risk characterization (EPA, 1992a, 1997). Conversely, toxicology studies can be designed to reflect the exposure pattern known to occur in a given subpopulation or occupational cohort.

Examples of exposure metric consideration include focusing on estimates of route-specific aggregate exposures (or absorbed doses) during a time period of interest (e.g., acute exposure) for comparison to route-specific single dose toxicological benchmarks (No-Observed-Adverse-Effect-Levels or NOAELs) such as acute neurotoxicity. In contrast, estimates of route-specific subchronic, time-averaged exposures should be compared to a NOAEL based on dose-related organ toxicity that only occurred following 90 days of repeat exposure by a relevant route (e.g., dermal).

Some chemical expressions of toxicity require repeated exposure (day after day) at a given level to exhaust an organism's capacity to compensate for biochemical imbalances or cellular injury (tolerance mechanisms). Other chemicals express toxicity by disrupting cyclical processes in an organism from a single exposure. Some toxicological manifestations may be related to the progression of related events (e.g., initiation of a genotoxic event and subsequent promotion) resultant from exposures at different stages of an organism's lifespan. Thus, a chemical's toxicological effects can be related to the exposure pattern (dose, frequency and duration) and associated absorption, metabolism, distribution and elimination kinetics. Historically this has been considered under the penumbra of toxicokinetics and toxicodynamics (see Fig. 1).

The purpose of this paper is to examine the toxicology requirements under FIFRA and explore approaches to improve the applicability of the toxicological data for occupational risk assessment either through changes in study design or interpretation. Many of the European requirements for testing pesticides are similar to FIFRA. Thus, several of the recommendations pertain to the EC, as well. We examined toxicology study designs, interpretation of

results, and attempted to establish a logical, relevant and scientifically credible basis for risk characterization.

DERMAL PHARMACOKINETICS AND PHARMACODYNAMICS

The dermal route is the primary route of exposure both for operators (mixers, loaders, and applicators; Wolfe, 1976) and re-entry workers (Fenske *et al.*, 1989). Those chemicals with very high vapour pressures, such as fumigants, are exceptions. Most FIFRA toxicology studies are conducted via the oral route. Although, dermal absorption studies are 'required for compounds having a serious toxic effect as identified by oral or inhalation studies for which a significant route of human exposure is dermal...' (Zendzian, 1994). Some means must be found to relate bioavailability of an absorbed oral dose from a toxicology study to workers' dermal exposure. Historically this has been addressed either by conducting a dermal absorption study (both OECD and FIFRA guidelines recommend the rat), or by conducting dermal toxicology studies. Both approaches have inherent difficulties.

The use of laboratory animals rather than humans to derive dermal absorption data raises questions about estimated human absorbed dose. Although chemical absorption by rat skin sometimes resembles that of human skin, more often it does not (Wester and Maibach, 1993). Comparison of dermal absorption of a dozen different pesticides by both rats and humans indicates the rat skin is more permeable to pesticides (Table 1). Thus, utilization of dermal absorption from laboratory animals will likely provide an inaccurate estimate of the absorbed human dose.

Without human dermal absorption data, it is not possible to correct quantitatively for the commonly observed differences in rat and human dermal absorption. A typical human dermal absorption study exposes an individual to nanograms of pesticide per kilogram bodyweight (Feldmann and Maibach, 1974). When a pesticide is used according to the label, exposure is predictable, unavoidable and in the vast majority of cases, benign (Krieger and Ross, 1993). There has been significant debate in the US about the ethics of purposeful application of pesticides to humans to determine dermal absorption (EPA, 2000). Given that dermal exposure will occur during and/or following normal pesticide use, it seems unethical *not* to quantify the amount of dermal exposure as a foundation for assessing and communicating the significance of exposure.

The ratio of human to rat *in vitro* dermal absorption has been used by regulatory agencies (Thongsinthusak *et al.*, 1992) as a quantitative means to estimate *in vivo* human dermal absorption from *in vivo* rat dermal absorption data, i.e.,

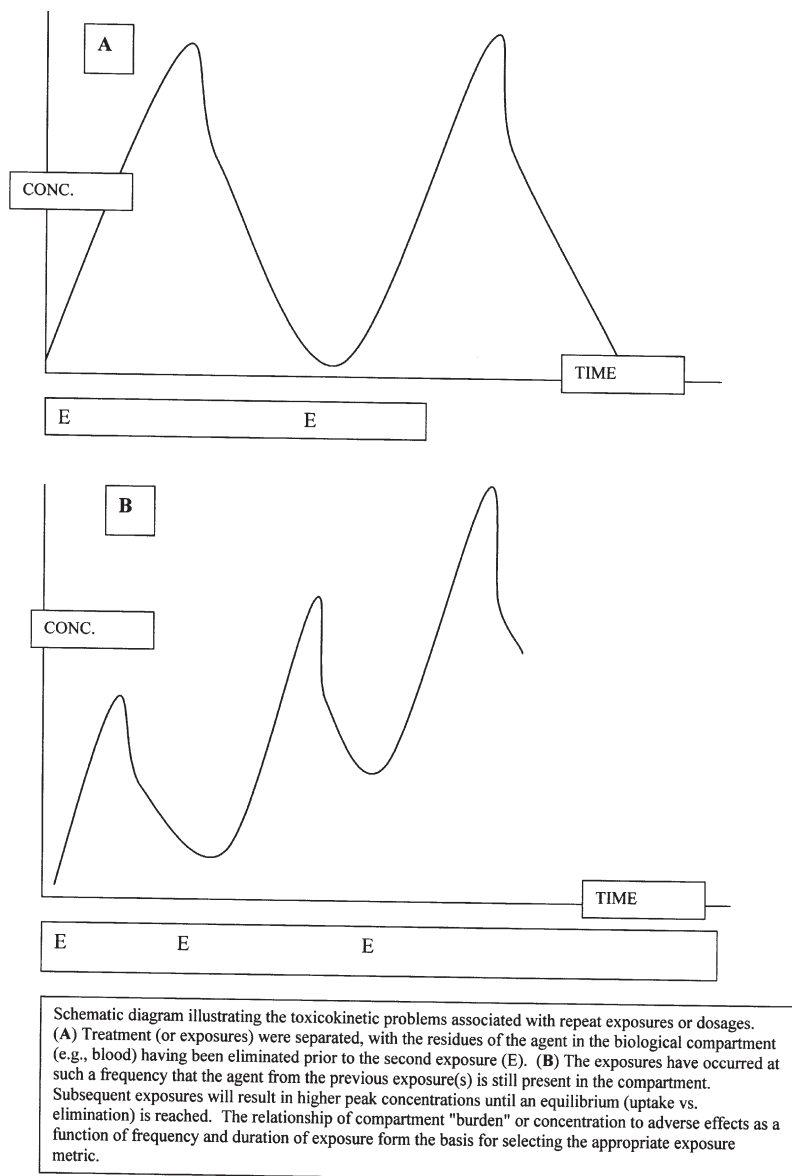


Fig. 1. Plasma kinetics as function of dose interval and depuration rate.

$$\text{in vivo}_{\text{human}} = (\text{in vitro}_{\text{human}} / \text{in vitro}_{\text{rat}}) (\text{in vivo}_{\text{rat}}) \quad (1)$$

OECD has proposed a similar use of *in vitro* data, but an expert panel evaluating *in vitro* dermal absorption data determined that there are currently insufficient data to validate this approach. Validation of the method to predict *in vivo* human dermal absorption from *in vitro* data awaits compilation of both *in vitro* and *in vivo* data from several chemicals obtained from rats and humans under comparable experimental conditions.

Animal dermal absorption and toxicity studies under FIFRA not only have the problem of not representing human dermal penetration, but also a different exposure metric than workers experience. In the

laboratory studies, the full dose of pesticide is placed all at once on the skin at the beginning of the exposure period. Dermal exposure in the work place occurs over an entire workday. Spencer *et al.* (1995) compiled results of worker exposure monitoring for hands of harvesters. The resulting data clearly indicate that although the rate of acquisition declines over time, levels continue to increase. Thus, a worker's dermal dose is accumulated over an 8-h period. This presents profound difficulties in interpreting dermal toxicity data generated in laboratory animals.

Recommendations for modification of dermal absorption study design

Current guidelines for dermal absorption studies do not require identification or characterization of pestic-

Table 1. In vivo dermal absorption of pesticides in rats and humans^a

Pesticide	Human absorption (%)	Rat absorption (%)	Ratio: rat/human
Atrazine	3	8 ^b	2.7
Azinphosmethyl	16	44 ^c	2.8
Chlorpyrifos	10 ^d	66 ^b	6.6
DEET	7 ^e	32 ^f	4.6
Diquat	1 ^g	3 ^h	3.0
Isofenphos	4 ⁱ	16 ^j	4.0
KBR 3023	4 ^k	23	5.8
Lindane	9	31	3.4
Malathion	6	28 ^l	4.7
Orthophenyl phenol	43 ^m	65 ⁿ	1.5
Parathion	10	95	9.5
Permethrin	2 ^o	28 ^p	14.0
Propoxur	20 ^h	50 ^q	2.3
Grand mean±SD	10±11	38±26	5.0±3.4

^aAdapted from those compiled by Wester and Maibach (1993), except as otherwise noted

^bShah and Guthrie (1983)

^cThongsinthusak *et al.* (1999b)

^dThongsinthusak and Krieger (1991)

^eSelim *et al.* (1995)

^fSnodgrass *et al.* (1982)

^gFormoli (1995)

^hFeldmann and Maibach (1974)

ⁱWester *et al.* (1992)

^jBrodberg (1990)

^kSangha *et al.* (1998)

^lDary *et al.* (1994)

^mTimchalk *et al.* (1998)

ⁿHughes and Hall (1995)

^oFormoli (1991)

^pThongsinthusak and Ross (1999)

^qSanborn (1994)

ide metabolites (Zenzian, 1994; De Heer *et al.*, 1999). If data on the metabolite distribution and recovery following dermal dosing were known, one could determine whether the difference in route results in a difference in metabolism. This information is crucial to understanding the predictive value of the oral toxicology studies for potential toxicity following dermal exposure. Additionally, having both dermal and oral pharmacokinetics are very helpful in constructing physiologically based pharmacokinetics for extrapolating between species (Dong *et al.*, 1996). For example, partition rates from blood to tissues may be measurable in an oral study but not in a dermal study due to detection limits, but absorption rate through the skin and subsequent excretion rate are critical in relating dermal dose to tissue concentration.

Absorbed dose in workers is most precisely measured using excretory metabolites. By quantifying human metabolites following dermal exposure, it may be possible to determine if there is a primary excretory metabolite that meets the criteria for biomonitoring in worker exposure studies (Woollen, 1993). Because there is a requirement to identify all major

metabolites following oral administration, the analytical standards for at least characterizing metabolites from the dermal route should exist at the time the dermal absorption study is performed.

Dermal absorption tends to be inversely related to dose density (Wester and Maibach, 1976). As dose density increases over a 50 to 100-fold range, percent dermal absorption usually (but not always) decreases 1.2-13 fold (Thongsinthusak *et al.*, 1999a). It should be noted that the distribution of dermal exposure is not uniform on a worker's body. The hands typically have several-fold higher dose density than other portions of the body (Table 2). The data suggest that hands receive one to three orders of magnitude more exposure per unit area than other regions of the body (Krieger, 1995). Consequently, the low and high doses in a dermal absorption study should bracket the estimated dermal dose density ($\mu\text{g}/\text{cm}^2$) workers are expected to receive.

The measurement of dermal absorption over the range of expected exposures may allow different absorption factors to be applied to various regions of the body. However, development of such factors may be complicated by the fact that in vivo dermal absorption of a given dose appears to exhibit regional differences over the body (Maibach *et al.*, 1971; Guy and Maibach, 1989; Ross *et al.*, 2000). At the present time, though, there are no scientific models that examine the effect of multiple concentrations of pesticides on the skin, separated spatially and/or chronologically, on the absorbed daily dosage (Wester and Maibach, 1993).

Finally, a dermal absorption study should be of sufficient duration to determine whether residues in and on the skin are bioavailable. Currently, skin residues remaining after washing the treatment site 8-10 h following dose application are assumed to be bioavailable at 24 h when many dermal absorption studies are terminated. An exponential saturation model for estimating dermal absorption was used to compare the calculated rat dermal absorption for five pesticides with and without the direct addition of bound skin residues (Thongsinthusak *et al.*, 1999b). The study indicated that the bound skin residues were frequently not bioavailable. As illustrated in Fig. 2, the asymptote of urinary and fecal excretion yields an estimate of 1.15% dermal absorption for mettam excluding tissue residues, while the standard FIFRA method would yield an estimate of >3.8% if the rats had been sacrificed at 24 h. Thus, studies terminated at 24 h are likely to be inaccurate regarding rat dermal absorption. Thongsinthusak *et al.* concluded that an optimally designed dermal absorption study should continue to collect excreta for seven days, or ten urinary excretion half-lives, whichever is shorter in order to most accurately estimate dermal absorption.

Table 2. Differences in dose density between hand and remaining body

Scenario	Hand (μg)	Hand ($\mu\text{g}/\text{cm}^2$) ^c	Remainder body (μg)	Body ($\mu\text{g}/\text{cm}^2$) ^c	Ratio hand/body
Mix/Load #3 ^a	28 400	29	163	0.0095	3000
Airblast #11 ^a	1230	1.2	2390	0.14	8.6
Groundboom #13 ^a	65	0.07	77	0.0045	16
Strawberry Harvester ^b	24 000	24	17 000	0.99	24

^aFrom PHED (EPA, 1998) assumed 10 lb handled per day

^bFrom Maddy *et al.* (1989)

^cHand area: 990 cm^2 ; body excluding hands and feet: 17 100 cm^2 (EPA, 1997)

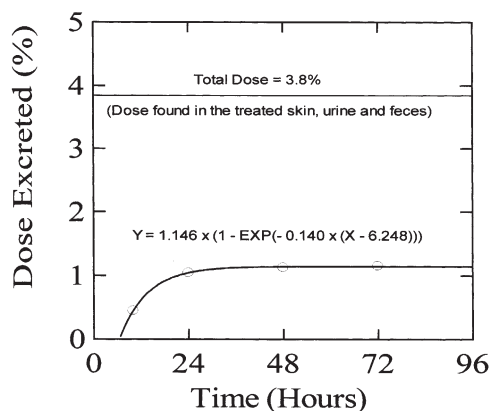


Fig. 2. Method to estimate bioavailability of bound skin residues and current FIFRA method: plots of cumulative excretion of metam-sodium excreted in urine and feces of rats following a topical dose of 8.6 $\mu\text{g}/\text{cm}^2$. [Bottom curve is fitted to cumulative excretion data (ovals) with an exponential equation obtained from iterative non-linear regression. Upper curve shows the value obtained using current FIFRA method.]

Recommendations for modification of dermal toxicity study design

Dermal toxicity studies in laboratory animals (21–90 days) are becoming more common, and may be more representative of the exposure dynamics and kinetics of workers' dermal exposure. However, several factors make this dermal toxicity data difficult to interpret. First, high dosages used in dermal toxicity studies are difficult to reliably reproduce. Much of a dermal dose is typically either applied to or soaked up by the wrapping that goes around the animal to protect the dose site. Second, the bioavailability of a dermal dose generally decreases with increasing dose. However, there are exceptions, and increased dermal dosing, occlusion of the dosing site or dermal irritation can result in significant increases in dermal absorption. Two examples for which the above phenomenon applies are boric acid and diquat (Wester *et al.*, 1998; Feldmann and Maibach, 1974). Third, there are finite limits of dose density that cannot be exceeded (~ 2 g/kg), because even with wrapping, the dose cannot be maintained at the site of application. As a consequence, for a pesticide with

relatively high use rates but low toxicity, it may not be possible to conduct a dermal toxicity study at levels sufficiently great to demonstrate an adequate Margin of Exposure. In some cases, it may not be possible to achieve an effect level from dermal dosing, making the NOAEL of questionable regulatory value.

ORAL PHARMACOKINETICS

Oral Absorption, Distribution, Metabolism and Excretion (ADME) studies are required to register most pesticides. These studies, if done thoroughly, permit estimation of the percent of administered dose absorbed, excretory metabolites, rate of excretion and tissue residue levels. This information can be a vital link for interpreting other oral toxicity studies, because it gives indication of residue levels in target tissues and potential for bioaccumulation, and the metabolite(s) that may be responsible for either intoxication or detoxification and where those conversions take place.

Comparative oral and dermal pharmacokinetics (Ross *et al.*, 2000; Schoenig and Osimitz, 2000) show that oral dosing typically produces peak plasma levels more quickly than dermal dosing and at a lower dose level for equivalent Area Under the Curve (AUC) as in Table 3. The results shown in Table 3 support the conclusion that dermal dose is inversely related to percent dermal absorption. The results also clearly indicate that oral absorption is much more efficient than dermal absorption. Most importantly, peak plasma levels following oral exposure are 1.4 to 149-fold higher than following dermal exposure in these examples. Additionally, from these examples it becomes apparent that it takes four to seven times longer to reach peak plasma levels following dermal exposure than following oral. Thus, dermal exposure translates to low plasma levels resulting from long-term absorption in workers exposed dermally. These plasma levels are not simulated in oral toxicology studies. Regardless of route, a bolus dose produces a higher plasma level at the target tissue than the same amount given over a longer interval. This has implications for the accuracy of risk assessment based on such NOAELs.

Table 3. Peak plasma levels in man after oral and dermal exposure to chlorpyrifos and fluazifop-butyl

Pesticide	Applied dose (mg) ^c	Route	Absorption (% applied)	Peak level (µg/l./mg)	Time to peak (h) ^d	Peak ratio oral/dermal
Chlorpyrifos ^a	41.7	Oral	72	22.3	6	–
Chlorpyrifos ^a	416	Dermal	1.4	0.15	24	149
Fluazifop-B ^b	6.1	Oral	100	100	3	–
Fluazifop-B ^b	200	Dermal	1.5	73.3	22	1.4
Fluazifop-B ^b	20	Dermal	3.4	32.4	22	3.1

^aAdapted from Nolan *et al.* (1984)

^bIn vivo absorption as measured by Auton *et al.* (1993) except for oral; note that the peak plasma concentration ratios between dermal and oral dosing would have been (proportionally) higher, if a value lower than the default of 100% were used for oral absorption of this pesticide

^cNormalized for total absorbed dose

^dIntervals between the time of dosing and the time at which the peak plasma level occurred

Pesticides with very short half-life (e.g., carbamates) that have reversible effects should be tested commensurate with exposure duration. For example, a dose of propoxur that would have produced evidence of severe intoxication in humans if given in a single oral bolus caused no observable effects when administered in divided doses spaced half an hour apart (Vandekar *et al.*, 1971). Also, rats exposed daily to a diet containing levels of propoxur exceeding the bolus LD₅₀ exhibited no mortality for a year on this regimen. Another example is oxamyl. In this case the subchronic oral cholinesterase inhibition NOEL is 21-fold *higher* than in the bolus acute neurotoxicity study, because the rats consumed the oxamyl-treated food at night, but were not tested for cholinesterase inhibition until the morning (EPA, 1999a).

Recommendations for modification of oral pharmacokinetics study design

ADME guideline studies typically require a single dose at a level producing no toxicity, a single dose at a level producing toxicity and 14 low doses followed by a single radiolabeled dose to determine steady state kinetics (EPA, 1996a). An intravenous or other parenteral route dosing regimen is also recommended if solubility and toxicity permit. However, this study is rarely conducted despite its utility in estimating oral bioavailability.

The low dose ADME study should approximate the *human* absorbed dosage. Human absorbed dosages of pesticides are typically in the range of ng/kg to µg/kg body weight (Krieger and Ross, 1993). FIFRA guideline low dose ADME studies are usually conducted at the NOAEL for adverse effects, typically in the milligram per kilogram range in laboratory animals. While this discrepancy in dose between typical human uptake and experimental animals may seem trivial, it can introduce significant uncertainty. With dose differences of up to three orders of magnitude between human experience and animal tests at the NOAEL, several factors can potentially confound

interpretation of toxicity data. Non-specific binding at low dose levels can influence systemic availability in humans resulting in even lower absorbed dose. Peak absorption may be enhanced or delayed at lower concentrations in humans. Dosages at the test animal NOAEL may saturate the primary detoxification pathway leading to alternate metabolites not present at lower dosages in humans.

As a toxic effect is normally related to the absorbed dose, an oral NOAEL should be corrected for gastrointestinal bioavailability. This requires either an intravenous, intraperitoneal, subcutaneous, or a biliary cannulation study. The normal transit time of food (and unabsorbed dose of orally administered pesticide) through the rat GI tract from mouth to anus is approximately 24 h to clear >80% of a bolus dose (Walsh and Ryden, 1984). Significant (>20% of dose) fecal excretion beyond 24 h is an indication of enterohepatic circulation, that represents absorbed dose. Evidence of absorption from an ADME study despite significant fecal excretion include delayed fecal excretion beyond 24 h, extensive fecal metabolites, and molecular weight or parent compound or metabolite >300 Da. These are all factors consistent with enterohepatic circulation (Williams, 1971).

TOXICOLOGIC EFFECT OF INTERMITTENT EXPOSURE

Although the exposure regimen (periodicity) in many pesticide toxicology studies is continuous, people are exposed in the workplace intermittently. Typically a worker is exposed a few days per week to an extensively used pesticide, and perhaps less than once per week for specialty use pesticides (hand harvested crops, nursery and residential applicators are notable exceptions). With the exception of inhalation toxicity studies, most studies feature daily exposure; whether by gavage, in drinking water, diet or dermal application. This difference in exposure regimens between humans and laboratory animals creates tremendous uncertainty in the assessment of hazard.

Both the exposure regimen in toxicology studies, and occupational exposure occur for only part (generally minutes to hours) of any given day. Thus, human and laboratory animal exposures are averaged or amortized over 24 h. In the case of inhalation toxicology studies, exposure occurs for 6 h on five of seven days, and that dosage is further amortized an additional 48 h. At some point amortization cannot be justified either for toxicology studies or occupational exposure assessment. This is largely dependent on two factors. One has to do with the amount of residual pesticide from one exposure to the next (half-life *in vivo*). The other has to do with the carryover of effect from one exposure to the next (reversibility).

Human exposure to pesticides can be viewed as a continuum. Exposure duration varies tremendously for the same pesticide depending on whether the individual is a grower, commercial handler, or a harvester of treated crops. Because of study design, toxicology studies are inherently of determinant duration. Occasionally effects are observed before study termination. More frequently, effects are observed at interim or terminal sacrifice periods predefined by the protocol. It is possible to estimate amortized exposure for any number of combinations and permutations of handling and/or re-entry work, and generally conclude that there will be subchronic exposure. However, it is up to the judgment of the risk assessor and the available toxicology data to gauge whether subchronic human exposure approximates laboratory animal studies of a week or three months duration. Likewise, acute toxicity studies may be hours or days in duration. By policy Cal/EPA's Department of Pesticide Regulation has defined subchronic human exposure as 30 or more exposure days in a 90-day period (Sanders, 1998). The 90-day period was chosen because that is the normal duration of a subchronic toxicity study, and may be related to human seasonal exposure. The issue of time to effect is generally not pertinent to chronic endpoints, because an estimate of annual or lifetime exposure is clearly defined as 365 days or 75 yr, respectively.

The primary difference in dose-response assessment between acute and chronic endpoints is the number of exposures that must occur before the effect can be observed. This time to effect is critical in differentiating type of response, and there are frequently limits on the minimum number of doses producing the effect (response). It is generally not possible to develop a chronic effect from acute or short-term exposure *unless* that exposure produces irreparable damage or the compound has an extremely long half-life (EPA, 1992b). Compounds in the former category would include direct acting genotoxic carcinogens, and mutagens capable of producing teratogenicity. Highly lipophilic compounds and heavy metals would likely fall in the latter category.

Time to effect is a critical factor, because the degree of exposure can change from one day to the

next. The longer the time to effect, the more a person experiences average exposure, since exposure fluctuates for a person from day to day and there is typically some carryover of exposure from one day to the next due to the dermal reservoir. Longer duration pesticide use patterns also typically produce more intermittent human exposure (and lower dosage) due to bad weather days, absences due to common illness, percent crop treated with a particular pesticide, regular days off, labor laws, equipment breakdown, chemical availability, environmental degradation, etc. Intermittent exposure may become so infrequent that there would be a low probability that residual pesticide or tissue damage would carryover from one exposure episode to the next.

Most pesticides have biologic half-lives well below 24 h (Feldmann and Maibach, 1974) meaning that less than 12% of the parent would be carried over to a second exposure 72 h later. For this reason averaging intermittent exposures over a period greater than approximately 72 h (three days) between exposures is generally inappropriate. In specific instances where half-life is very long the re-exposure interval could be greater than three days for amortization purposes, but this would be very rare. In general, if there are not at least 30 exposure days over a 90-day interval, or at least 120 exposure days in a year, calculation of subchronic or chronic exposure, respectively, is misleading. Averaging human exposures with large time lapses between exposures fails to take this into account. Gauging infrequent human exposure with laboratory animal toxicity studies based on daily dosing lacks relevance.

Even on an acute basis, occupational exposures tend to be intermittent. An example of small, divided exposures is the experience of operators that dust date palms with malathion. In this case exposure occurs over an entire workday, and mg/kg absorbed dosages are observed (Krieger and Dinoff, 2000). These dosages are clearly in the range that when delivered as a bolus to either humans (Krieger and Dinoff, 2000) or laboratory animals, they produce significant cholinesterase inhibition. However, the operators exhibited no evidence of cholinesterase inhibition when measured after a full day of malathion exposure. This may have been due to the rapid hydrolysis of succinic acid esters in malathion which produces a water soluble metabolite that is excreted and does not react with cholinesterase. Additionally, dose acquisition over several hours coupled with slower dermal than oral absorption could be responsible.

The kinetics of chlorpyrifos with an excretory half-life of ~27 h (Nolan *et al.*, 1984) suggest that it is cleared from the body more slowly than malathion with an excretory half-life of ~16 h (Dary *et al.*, 1994). As a result of the longer half-life, there is more carryover of pesticide in the body from one exposure to the next, and intermittent exposure can legitimately be amortized over a longer period between exposures.

However, even for compounds with intermediate half-life, it is inappropriate to apply results of subchronic toxicity testing with daily dosing to human exposures occurring 1–2 times per week over a 90-day period. Under these intermittent conditions, either an acute endpoint should be applied, or a toxicology study should be designed to match the intermittent nature of exposure.

An example of the ‘Haber Principle’ of toxicology which says that a given product of exposure time and dosage will produce the same effect over a limited range of times and concentrations (Atherley, 1985) can be seen in Table 4. Molinate, a thiocarbamate herbicide, produces neurotoxic effects in rats. As the number of doses increase, the dose level required to produce the effect (or no effect) decreases. High doses for short durations produced frank clinical signs. Longer exposures at lower doses produced histopathologic changes consistent with those signs. Since the agricultural use season is approximately six weeks in length, and one-third of those days are too windy to apply molinate, the most appropriate toxicology study duration for risk assessment is 4 weeks or less of continuous rat dosing.

As human exposure tends to be intermittent, it is generally amortized (averaged) over episodes of non-exposure, in order to derive an exposure dose whose risk can be gauged by a laboratory animal study of similar duration. This amortization is an implicit use of Haber’s Principle. Clearly, the length of time between exposures cannot be so long that there is no residual toxicant from the last dose before the next dose is received, or alternatively that the effect produced by the last dose has been completely recovered (Rozman and Doull, 2000).

On the other hand, effective single exposure/single dose oncogens are not uncommon (Calabrese and Blain, 1999). Genotoxic carcinogens can and should be administered over a significant portion of the lifetime to mimic occupational exposure. But occupational exposures to pesticides typically occur over

one’s working life (and the life of the pesticide in the marketplace). The use of an amortized Lifetime Average Daily Dosage to approximate lifetime exposure from intermittent doses of a chemical may underestimate risk 2 to 5-fold, but is more likely to overestimate it by several orders of magnitude (Murdoch *et al.*, 1992; Murdoch and Krewski, 1988; Kodell *et al.*, 1987; Morrison, 1987).

Recommendations for modification of toxicology study design to incorporate intermittent exposure

Dose intermittence becomes an issue in designing and interpreting toxicology studies as one progresses from acute to chronic study regimens. The terms ‘chronic’ and ‘acute’ need to be distinguished because there is confusion about whether chronic and acute refer to exposure duration (time) or nature of the response (permanence of effect). A permanent teratogenic effect can result from a single exposure that most call an acute (or short-term) exposure. Others call a teratogenic effect chronic because the effect is long term. It is not possible to have a chronic exposure without first having an acute exposure, although it is possible to develop chronic effects for several toxicologic endpoints from an acute exposure. Thus it would seem reasonable to clearly distinguish exposure duration from duration of effect. The issue has considerable importance in scientific assessment of risk as well as in risk management and particularly in risk communication.

There are several study types an acute toxicologic endpoint could be based upon that would cover a significant percentage of existing compounds. Currently, acute neurotoxicity tests cover many insecticides including organophosphates, carbamates, pyrethroids, nicotinoids and GABA agonists. Thus, an acute toxicity test could be based upon the acute neurotoxicity study design with relatively little modification. As indicated above, developmental toxicity testing, when it results in effects on offspring, is frequently utilized as an acute toxicity test.

For short-term exposure, a routinely conducted toxicology study with dosing duration of 14–21 days (developmental) coincides with the duration of many types of worker exposure. Adverse effects occurring from two or three weeks of dosing, are likely to be found with this regimen. It is not unusual (although not required) to do a 45-day interim sacrifice in a 90-day subchronic study. Thus, there are normally opportunities to observe histopathologic evidence of adverse effect at exposure intervals <90 days with these various tests.

Toxicity studies should be designed to mimic worker exposure duration. In cases where exposure occurs over an extended period, especially with rapidly cleared pesticides, toxicology study design could be modified to mimic the manner of human exposure. That is continuous administration using techniques such as osmotic pumps (Alzet, 2000) for dose deliv-

Table 4. The Haber Principle illustrated with rat toxicology data from molinate^a

Test type	Toxic effect	Time to effect (days)	NOAEL (mg/kg)	LOAEL (mg/kg)
Neurotoxicity	Clinical signs	7	15	75
Neurotoxicity	Brain struct. Δ^b	28	2	5
Neurotoxicity	Nerve degen.	365	1.9	17
Neurotoxicity	Nerve degen.	730	NS ^c	0.3

^aFrom Cochran *et al.* (1997)

^bBrain structural changes

^cNS: not seen (NOAEL not established)

ery should be considered for acute and intermediate term toxicology studies. Such dosing would mimic the extended period of human dose acquisition (8+ h) and subsequent dose absorption over many additional hours.

Many pesticides are in the marketplace less than a working lifetime of up to 40 yr, due to patent life, competing products, pest resistance, environmental concerns, regulatory cancellation, etc. Animal toxicology dosing duration could be adjusted accordingly. That is, laboratory animals could be dosed daily for 1/4 to 1/2 of their life expectancy and then necropsied at normal life expectancy. A single group of animals dosed at the estimated Maximum Tolerated Dose on this regimen would help to resolve the effect of intermittent exposure at relatively little additional cost.

EPA oncogenicity guidelines allow threshold calculations for epigenetic oncogens. Clearly, a peroxisomal proliferator or other non-genotoxic oncogen would require a number of exposures to produce an effect, and the effect would unlikely result from intermittent exposure, unless the exposures were massive. Thus the averaging time or amortization of dosing time and times between exposures should have finite limits to consider them to act in concert.

Unless there is a clear mechanism, the presumption that acute or intermittent short-term/subchronic exposure can produce chronic effects may not be accurate. On the other hand, given a sufficiently high dose there will probably be an effect from acute exposure. As a result an acute NOAEL should always be determined. A subacute or subchronic (short or intermediate term) NOAEL should be determined if anticipated exposures of workers warrants *and* there is an effect of concern that manifests from short-term exposure.

Interspecies pharmacokinetic studies are suggested as a means to determine which toxicology study dosing regimen best reflects the intermittent exposure of workers. The longer a pesticide's half-life, the more doses are required to reach a steady state in plasma. For pesticides with long half-lives, intermittent exposure will not influence steady state plasma levels as greatly as for pesticides with short half-lives. Thus, the shorter the half-life, the shorter the duration of the toxicology study relevant to worker exposure.

INHALATION TOXICITY/PHARMACOKINETICS

Although the dermal route is the primary occupational exposure route for most pesticides, there are some exceptions. These include pesticides with very high vapour pressure (typically >1 Pa), such as fumigants. Fumigants exist largely or exclusively in the vapour state, so the inhalation route assumes primary importance. Also, for compounds with very low (<1%) dermal absorption, small inhalation exposures can be the primary source of absorbed dosage because inhalation absorption is generally much more efficient

than dermal. Finally, certain specialized application equipment is designed to generate aerosols <20 μ mass median diameter (mmd) that are respirable (e.g., thermal foggers).

FIFRA requires a subchronic inhalation study 'if use may result in repeated inhalation exposure at a concentration likely to be toxic'. Acute inhalation studies are required 'if the product consists of or under conditions of use will result in an inhalable material (e.g., gas, volatile substances, or aerosol/particulate)' (CFR, 1998). These inhalation studies are conducted on virtually every active ingredient in a standard protocol where animals (usually rats) are placed in chambers into which a controlled atmosphere of test substance is introduced. Under these conditions, the whole body is exposed to the test compound, not just the respiratory tree. As a result, significant deposition can, and does occur to the skin and fur of the animal. Because of grooming and dermal absorption, this study design frequently produces absorbed dosages 2 to 18-fold greater than nose only inhalation exposures (Langard and Nordhagen, 1980; Wolff *et al.*, 1982; Iwasaki *et al.*, 1988; Jaskot and Costa, 1994; Tyl *et al.*, 1995).

Further complicating interpretation of inhalation toxicity studies, is the size of particulates typically generated for the study. Because most pesticides have low volatility it is difficult to generate stable atmospheres of particles in the mass range normally encountered in occupational environments. To facilitate production of uniform atmospheres for testing, and to insure delivery to the deep lung, particles are normally created in the <10 μ mmd range (EPA, 1988). Some pesticides are exquisitely toxic following deposition in the deep lung, e.g., paraquat (Wyatt *et al.*, 1981). However, particulates <10 μ are typically <8% of the total mass of dust generated by harvesting or tilling machinery (Atiemo *et al.*, 1980). Respirable particulates on leaf surfaces are typically <10% of the total with a median of 4.4% (Popendorf *et al.*, 1982). Particulates generated by agricultural and pest control operators equipment are generally >90% by mass in the size range larger than 30 μ . This means that most of the potential exposure mass is falling downward faster than they can move sideways into the personal breathing space and the respiratory tract (Hayes and Laws, 1991). As a result, an extremely small percentage of the pesticide mass in air is available to areas of the exposed worker's respiratory tree below the larynx.

Inhalation studies with a variety of gases and solvent vapours have shown that uptake in humans (Astrand, 1975; Raabe, 1988) is unlikely ever 100% (Table 5). Two of the chemicals in Table 5 are pesticides in their own right, and several of the remaining chemicals are found in pesticide formulations. Chemicals with low molecular weight and high reactivity or water solubility tend to be most efficiently

Table 5. Lung uptake of organic and inorganic vapours in humans

Chemical	Uptake/retention (%)
Acetone ^a	31
Aliphatic and aromatic white spirit ^b	59
Benzene ^{a,c}	54
Chloroform ^c	46
Formaldehyde ^c	75
Ethyl acetate ^a	60
Ethyl alcohol ^a	33
n-Hexane ^a	28
Mercury vapour ^d	73
Methyl t-butyl ether ^c	40
Methyl bromide ^c	55
Methylene chloride ^b	42
Octamethylcyclotetrasiloxane ^f	12
Styrene ^g	65
Toluene ^a	53
Trichloroethylene ^h	56
Grand mean±SD	49±17

^aNomiyama and Nomiyama (1974)

^bAstrand (1975)

^cRaabe (1988)

^dHursh *et al.* (1980)

^eAmberg *et al.* (1999)

^fUtell *et al.* (1998)

^gPezzagno *et al.* (1985)

^hMean of determinations from references a, c, Astrand and Orvum (1976); Bartinocek (1962); Monster *et al.* (1976)

absorbed following inhalation, but even these rarely exceed 70% uptake.

Recommendations for inhalation toxicity study design

Whenever possible one should use nose-only rather than whole-body inhalation study designs to reduce dosing from non-respiratory routes. Although the nose-only method is not always possible due to the additional stress it places on the test animals (e.g., nose-only developmental toxicity studies may be ill advised), it is a viable alternative for most acute and subchronic exposure regimens. When interpreting results of whole-body inhalation studies, one must either attempt to discount the study for use in risk assessment, or alternatively try to estimate the total absorbed dose (not just the amount theoretically inhaled based upon atmospheric concentration and respiration rate) as in Cochran *et al.* (1997).

For practical experimental toxicology reasons, it is usually difficult or impossible to conduct an inhalation study without generating a predominance of sub 10 µ particles. This is likely the reason that guidelines for conduct of inhalation toxicity studies require the generation of particles in the 1–4 µ range (EPA, 1996b). However, when applying the results of such a toxicity study, it is important to estimate the fraction of the human inhalation dosage in the sub 10 µ range. Only the dose in the sub 10 µ range should be used to juxtapose with the NOAEL from the animal toxicology study.

The balance of the human inhalation exposure (particles >10 µ mmd) should be added to the oral or dermal absorbed dose, since most of the dose deposited along the upper respiratory tract is eventually either expectorated or swallowed (EPA, 1999b).

Similarly, for vapour exposure, the inhalation dose should be reduced by the estimated lung uptake to reflect absorbed dose. In most cases compound-specific uptake is not known, and a reasonable default in the absence of data is 50%. In rare instances, both experimental animal and human uptake are known (e.g., for methyl bromide) and it is possible to differentially correct animal vs. human uptake. However, in most cases the default inhalation uptake should be applied to both animal and human data resulting in no differential uptake.

CONCLUSIONS

The toxicology studies required by law for registration of most pesticides emphasize the oral route of exposure. Regulators must use results of these toxicity studies to assess the risks of pesticide exposures in occupational settings, even though workers are exposed through different routes. Unless the risk assessor is aware of the differences, limitations and assumptions involved in generating both the toxicology NOAEL and the worker exposure estimates, an inaccurate portrayal of occupational risks will result.

Several areas in toxicology study design and interpretation could be improved to better reflect the types of exposures that workers receive. These modifications can frequently be made at little, or no additional expense, and would significantly improve the accuracy of risk characterizations. Some of the recommendations are:

1. Characterize the bioavailability of bound skin residues in laboratory animals. This can be accomplished by collecting excreta until the excretion curve plateaus (~10 half-lives or 7 days, whichever is shorter) in one extra group of animals at the lowest dermal dose tested. This will more accurately characterize dermal absorption in laboratory animals if dermal absorption studies in humans are not available.
2. Characterize urinary metabolites collected in dermal absorption studies. Such information is valuable in validating common disposition by the oral route, in physiologically-based pharmacokinetic estimates of delivered dose, and for identifying candidate metabolites for biomonitoring.
3. Consider the estimated worker exposure as the basis for dose selection in oral and dermal pharmacokinetic studies. The absorbed doses from occupational exposures are frequently less than the lowest administered in an oral pharmacokinetic

study. As current regulations allow discretion in the selection of the low oral pharmacokinetic dose, a preliminary worker exposure assessment could be used to aid in the selection of this dose. The pharmacokinetics at this dose level would be a more accurate representation of what occurs when workers are exposed.

Such a preliminary worker exposure assessment could also be used to estimate dose density on hands or a part of the body which would be anticipated to have highest exposure in a given scenario (e.g., head in airblast application). The highest estimated worker dermal dose density could be used as the high dose in a dermal absorption study. The lowest dermal absorption study dose could be one that approaches the average dose density of the remaining body surface of workers. The rationale for bracketing the range of expected worker exposure stems from the observation that dermal absorption is an inverse function of dermal dose density.

4. Utilize data from oral, dermal, and possibly inhalation pharmacokinetic studies to adjust estimates of pesticide bioavailability from toxicology studies and worker exposure monitoring studies. Estimating oral bioavailability may necessitate parenteral dosing which is suggested in guidelines for conducting pharmacokinetic studies, but not required.
5. Because inhalation toxicity studies of particulates require exposing laboratory animals to 1–4 μm particles, the resulting inhalation NOAEL is really only applicable to the fraction of the worker inhalation dose in the 1–4 μm range (typically <10% of the total inhalation exposure). The remaining ‘inhalation’ dose is really oral and should be compared to the oral NOAEL. Vapour exposure uptake, when available, should be used to correct both inhalation NOAEL and worker exposure monitoring data. Vapour exposure is only significant for high vapour pressure pesticides (>1 Pa). A default vapour uptake in the absence of data would be 50%.
6. Gauge the risk of worker exposure by toxicity studies of similar duration. As all work tasks produce acute exposures, a standardized acute (single dose) toxicity study should be developed based on those routinely required for neurotoxic pesticides.
7. Consider the differences in toxicokinetics and toxicodynamics between the laboratory animal studies utilized for regulatory endpoints and the intermittent and primarily dermal exposures experienced in the occupational setting when assessing the risks. Pharmacokinetic modeling of plasma levels following oral and dermal exposures should be compared with plasma levels following intermittent exposures in laboratory animals which mimic occupational exposure experience. This may require the development of new techniques

involving the use of osmotic pumps, dermal application or intermittent dosing regimens.

8. Use caution in gauging occupational exposures amortized across large time spans with chronic NOAELs. Haber’s Principle, the usual justification for such amortization, may not be applicable in the absence of cumulative tissue damage, or a long somatic half-life for a pesticide. For the manufacturer, testing the effect of intermittent exposure on the toxicological outcome may be a less costly alternative to accepting the estimated risk from continuous dose toxicology data. In the case of chronic toxicology testing, treating one group of laboratory animals for 5 days per week for 1/4 to 1/2 of their life followed by a recovery period constituting the remaining life expectancy might be a viable approach to determining the effect of intermittent exposure.

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