A Toxicokinetic Model to Assess the Risk of Azinphosmethyl Exposure in Humans through Measures of Urinary Elimination of Alkylphosphates

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Azinphosmethyl (APM) is one of the most common insecticides used in fruit farming. The object of this paper is to develop a quick and practical test for assessing the risk for humans coming into contact with APM. It has been shown that the principal component of occupational and/or accidental exposure is through the skin (C. A. Franklin et al., 1981, J. Toxicol. Environ. Health 7, 715–731), but our approach is applicable to exposures via any route or a combination of routes. The method proposed in the present paper can accommodate a single-event exposure or repeated exposures over long periods. Urinary alkylphosphate (AP) metabolites are reliable bioindicators of the presence of APM in the body; they are easily accessible and can be used to estimate APM body burden. We developed a simple toxicokinetic model to link the time varying APM body burden to absorbed doses and to rates of elimination in the form of AP urinary metabolites. Using this model and data available in the literature, we are able to propose a “no observed adverse effect level” (NOAEL) for APM body levels and for corresponding absorbed doses. We have established that after a single exposure, the safe limit corresponding to the NOAEL is reached at a cumulative 0.215 μmoles AP/kg bw eliminated in urine in the first 24 hours following the beginning of exposure. For repeated daily exposures at steady state, the corresponding urinary AP metabolite level is equal to a cumulative 0.266 μmoles AP/kg bw eliminated per 24 hours.

Key Words: azinphosmethyl; alkylphosphate; risk assessment; NOAEL.

The most common chemical substances responsible for severe poisoning among agricultural and horticultural workers, professional and amateur, are anticholinesterase insecticides: esters of phosphoric or phosphorothionic acid often called organophosphates (OP) and esters of carbamic acid (Ecobichon, 1995). A quick and ready test for assessing the exposure to those substances, and the associated risk for humans, would be of great help to medical staff involved in risk prevention or in the treatment of exposed persons. The test we propose is adapted to azinphosmethyl (APM), an ester of phosphorothionic acid, one of the most used insecticide in the world.

Like other OP insecticides, APM is a neurotoxicant and acts by poisoning the nervous system of target insects. Unfortunately, its action is not restricted to insects, and it can cause similar effects in higher forms of life. Its toxicity lies mainly in its instantaneous inhibition of the nervous tissue acetylcholinesterase (AChE), the enzyme responsible for the destruction and termination of the biological activity of the neurotransmitter acetylcholine (ACh). Inhibition of AChE results in an immediate accumulation of free unbound ACh at the ending of all cholinergic nerves, leading to continual stimulation of electrical activity (Koelle, 1994). There are four anatomical classes of cholinergic nervous fibers: postganglionic parasympathetic fibers, preganglionic fibers (both sympathetic and parasympathetic), motor fibers to skeletal muscle, and certain fibers within the central nervous system (CNS). All cholinergic fibers contain, at their terminals, high concentrations of ACh and AChE. Since these nerves play an important role in the normal functioning of the neuromuscular, central nervous, endocrine, immunological, and respiratory systems, AChE inhibition can impair their function. In mammals that have experienced an acute exposure, the symptoms appear as soon as the ACh overflow in the synapses is at a sufficient level to impair normal physiological function. Koelle (1994) has shown that with 50% AChE inactivation, some adverse physiological effects are observed. As soon as the local concentration of the OP begins to fall, reversal of effects commences. (Koelle, 1994).

In humans, as in animals, depending on the exposure dose to OP insecticides, the observed response varies from absence of effects to severely acute and chronic health damage (Sidell, 1994). The measure of erythrocyte acetylcholinesterase (RBC-AChE) in the blood, a single enzyme analogous to the AChE found in nerve tissue, correlates with clinical toxicity in the nervous system (Kaloyanova and El Batawi, 1991; Coye et al., 1986a; McCurdy et al., 1994; Jeyaratham and Maroni, 1994; Richter et al., 1992; Sidell, 1994). According to Zavon (1965), RBC-AChE is the best indicator of AChE activity at the nerve synapse, since it closely parallels the level of AChE in the brain.
central and peripheral nervous systems (CNS and PNS). Since blood is the route of transportation of APM to organs, it is expected that a depression of RBC-AChE will correlate with effects due to a rapid depression of AChE enzymes found in other tissues, both being inhibited by APM. Indeed, this correlation tends to increase as the speed of inhibition is rapid. For instance, in previously unexposed individuals, clinical signs of intoxication generally appear at inhibition of 60–70% of RBC-AChE. After sudden exposure, light clinical signs and symptoms were observed for a depression of RBC-AChE activity between 30% and 60%, especially if depression occurred rapidly (Coye et al., 1986a and b; Kaloyanova and El Bataw, 1991; Richter et al., 1992; Jeyaratham and Maroni, 1994; Sidell, 1994).

In chronic moderate exposure, the correlation between RBC-AChE reduction and toxic effects is weaker: workers may exhibit 70% to 80% RBC-AChE inhibition without manifesting apparent cholinergic symptoms, due to a tolerance phenomenon. It has been demonstrated in numerous animal studies that repeated exposure to OPs renders mammals less susceptible to the toxic effects of the acetylcholinesterase inhibitors, even though cholinesterase activities are often below normal (review by Sultatos, 1994). They explained this phenomenon by a down-regulation of cholinergic receptors due to the presence of an excess of agonists. Chronic inhibition of AChE as a result of exposure to an OP is thought to result in higher than normal levels of ACh (agonist) within a synapse, ultimately leading to down-regulation of postsynaptic receptors. Consequently, a given concentration of ACh at that synapse causes less response, since fewer receptors are available. However, this tolerance adaptation to a reduction in RBC-AChE and other AChEs does not contradict the link found between APM body burden and AChE reduction, as is observed in acute exposure. The adaptive response is more related to a tolerance to a relatively high level of AChE at the postsynaptic receptors (toxicodynamic response), in spite of the depression of RBC-AChE resulting from a given OP body burden (toxicokinetic response).

APM is a dimethyl OP pesticide, a derivative of dithiophosphoric acid, which is eliminated from the body in the form of metabolites formed in the liver. Considering the metabolism of OP compounds on the P = S and P-S-C bonds, it is expected that APM produces the following three dimethylated alkylphosphates (AP) metabolites: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethylldithiophosphate (DMDTP) (Coye et al., 1986c; Drevenkar et al., 1991). These three AP metabolites are excreted in urine, and recently their measurement was used as a method to confirm absorption of dimethyl OP pesticide. Indeed, even with no significant depression of RBC-AChE (less than 15% of the pre-exposure values), Franklin et al. (1981) found a high correlation between 48-h DMTP urinary excretion and the amount of active APM sprayed ($r = 0.77; p < 0.01$). McCurdy et al. (1994) have shown that the measure of the three urinary metabolites DMP, DMTP and DMDTP was the most sensitive indicator of exposure to APM. They have shown that even at levels below the one sufficient to produce adverse health effects, AP metabolites in urine correlate well with RBC-AChE decreased activity ($r = -0.77; p < 0.0001$). Aprea et al. (1994) showed good correlation ($r = 0.788$) between the hand-wash data and urinary excretion of the sum of the three AP metabolites, DMP, DMTP and DMDTP, in workers exposed to APM and chlorpyrifos-methyl.

Considering the above, it appears that the APM body burden is a good determinant of physiological effects. It is of interest to use the data available in the literature to link together absorbed dose, body burden, and metabolite excretion rates. Toxicokinetic models provide the framework to establish these links in the dynamic context of time varying dose exposure, body burden, and excretion data. In particular, we seek to estimate APM body burdens, either from dose magnitudes and sequences, or alternatively, from the time evolution of the excretion of a sensitive marker.

Some toxicokinetic models have been proposed concerning certain OPs (Nolan et al., 1984 for chlorpyrifos; Rabovsky and Brown, 1993 for malathion). However, no attempt has been made, so far as we know, to link, through a model, the time variations of doses, body burdens, and excretions. We propose a simple toxicokinetic model of OP absorption through single or multiple routes and elimination by possibly several pathways. This is an empirical black box approach, without details of the physiological mechanisms, yet it captures the principal features of the disposition of APM. The body burden built up is shown to depend strongly on the time sequencing of doses and on the opposing absorption and elimination mechanism.

It is well established that occupational or environmental exposure to OP is mainly due to dermal exposure; the latter usually accounts for more than 90% of the absorbed dose (Aprea et al., 1994). Hence, we concentrate here on application of the model to dermal exposure to APM and the use of the easily accessible urinary AP metabolites, as elimination markers, without precluding elimination by other unobserved pathways.

To make the model applicable to the situation at hand, one needs to estimate the absorption and elimination rates and the fraction of APM eventually eliminated through the observed pathway. The controlled experiments of Feldmann and Maibach (1974), with both iv and dermal exposure of volunteers, provide the data to estimate the free parameters of the model. Once these parameters are established, it is possible, in principle, to reconstruct the amount of APM absorbed and the total body burden at any time, past or future, from a single measure of total metabolites excreted in urine over a specified time period.

McCurdy et al. (1994) measured, in a group of orchard workers, both the excreted AP metabolites and the levels of decrease in RBC-AChE activity. Since no clinical effects were observed, their data can reasonably be used, in association with
the model, to determine a “no observed adverse effect level” (NOAEL). Using the links provided by the model between metabolite-excretion rates, APM body burden, and absorbed dose, we can estimate the body burden and the daily dose absorbed by these workers. This leads us to propose a NOAEL for doses of APM absorbed through the skin for the situations most often encountered: a single-day exposure dose or repeated daily doses. For these two situations, we also propose a simple test to assess if the NOAEL was reached, based on measures of cumulative urinary excretion of AP metabolites. This test meets the key criteria of being easily collected and having high degrees of specificity, and sensitivity.

**METHOD AND MODEL PRESENTATION**

Feldmann and Maibach (1974) studied the urine excretion of radiolabeled $^{14}$C-guthion, (Guthion is a trade name of APM) in six healthy volunteer males after intravenous (iv) administration as well as after skin application on forearms. Urine was collected for the first five post-application days, for both experiments. The $^{14}$C in urine was determined in 8 specimens per subject, each specimen containing a collection for a specified period of time. The first day was divided into three 4-h periods followed by a 12-h period. For the remaining four days, urine was collected as 24-h specimens.

It is known that in a first order system, the elimination of an iv dose can be represented by:

$$B(t) = \text{dose}_{iv} \cdot e^{-k_{\text{elim}} \cdot t}$$

where $B(t)$ represents the body burden at time $t$ post administration. This can be conveniently cast as a linear relation:

$$\ln B(t) = -k_{\text{elim}} \cdot t + \ln(\text{dose}_{iv})$$

A first order kinetic model implies that, at any time post exposure, the amounts excreted per unit of time in the form of a particular bioindicator, are proportional to the instantaneous body burden:

$$\frac{dO(t)}{dt} = k_o \cdot B(t)$$

where $O(t)$ stands for the observed cumulative urinary excretion as APM metabolites and $k_o$ is the corresponding proportionality constant. Note that if other elimination routes exist, than $k_o < k_{\text{elim}}$. Using the latter two equations we can write:

$$\ln \left( \frac{dO}{dt} \right) = -k_{\text{elim}} \cdot t + \ln k_o + \ln \text{dose}_{iv}$$

Thus, a first order kinetic model implies that the semilog plot of metabolites excreted per unit of time should have a slope identical to $\ln(B(t))$ for an iv dose. The basis for a first order model is well supported here by the observed data of Feldmann and Maibach (1974). The logarithm of the mean value of urinary metabolite concentrations for each time period, fitted with a regression line, shows a coefficient of correlation of 0.992 (Fig. 1). This prompts us to assume that a first order kinetic model for elimination is warranted.

The proposed kinetic model is presented in Fig. 2 (for symbol definitions, see Table 1). The system of equations that describes the model is:

$$\frac{dA_j(t)}{dt} = D_{\text{abs},j} \cdot A(t) - k_{\text{abs},j} \cdot A_j(t); \quad j = 1, \ldots M$$

where $j$ is a specific route of absorption and $M$ is the number of routes of absorption.

$$\frac{dB(t)}{dt} = \sum_{j=1}^{M} \left( k_{\text{abs},j} \cdot A(t) - (k_o + k_j) \cdot B(t) \right)$$
TABLE 1
Variables and Parameters of the Kinetic Model

<table>
<thead>
<tr>
<th>Item</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>D_{exp}(t)</td>
<td>Dose per unit of time of APM in contact with the body at site j (skin, lungs or digestive tract) at time t</td>
</tr>
<tr>
<td>D_{abs}(t)</td>
<td>Dose per unit of time of APM absorbed in the body via the route of contact with APM at time t. D_{abs}(t) = f_{abs} \cdot D_{exp}(t)</td>
</tr>
<tr>
<td>A(t)</td>
<td>APM burden in absorption site at time t</td>
</tr>
<tr>
<td>B(t)</td>
<td>APM body burden at time t</td>
</tr>
<tr>
<td>O(t)</td>
<td>APM burden accumulated in urine at time t as AP</td>
</tr>
<tr>
<td>N(t)</td>
<td>APM burden accumulated at time t in the non observed excretion route</td>
</tr>
<tr>
<td>W</td>
<td>Weight of a person in kg</td>
</tr>
<tr>
<td>f_{abs}</td>
<td>Fraction of the exposed dose that is bioavailable for absorption in the body at exposure route j</td>
</tr>
<tr>
<td>k_{abs,31}</td>
<td>Transfer rate constant (hr^{-1}) to the blood from a specific absorption site j</td>
</tr>
<tr>
<td>k_{o}</td>
<td>Excretion rate constant of APM in urine as AP (hr^{-1})</td>
</tr>
<tr>
<td>k_{elim}</td>
<td>Excretion rate constant of APM body burden from observed and non observed excretion routes k_{elim} = k_{o} + k_{elim} (hr^{-1})</td>
</tr>
<tr>
<td>k_{n}</td>
<td>Excretion rate constant of APM from a non observed route</td>
</tr>
<tr>
<td>N(t)</td>
<td>Non-observed cumulative excretion of APM</td>
</tr>
</tbody>
</table>

\[
\frac{dO(t)}{dt} = k_{o} \cdot B(t) \\
\frac{dN(t)}{dt} = k_{n} \cdot B(t)
\]

where N(t) stands for the non-observed cumulative excretion of APM, and k_{n} is the corresponding proportionality constant:

1. The values of k_{abs,31}, k_{o}, and k_{n} are determined using iv-observed data by Feldmann and Maibach (1974) in conjunction with the dynamics of the model.
2. The values of k_{abs,31} and f_{abs} for skin exposure are determined using the skin application experiment of Feldmann and Maibach (1974) and the instantaneous links between variables provided by the model.

The system of equations linking the state variables \{A(t), B(t), O(t), N(t)\} to the inputs \{D_{exp}(t)\} and the various parameters can be solved. Of particular interest to us is the relation thus established between the evolving APM body burden, B(t), and the cumulative observable APM excretion, O(t), in urine in the form of AP metabolites.

**Determination of Exchange Rate Constants**

**First Step: Determination of the Elimination Rate Constants k_{o}, k_{elim} and k_{n}**. For a single iv dose, O(t) depends only on the magnitude of that dose and the constants k_{elim} and k_{n}. Values of these rate constants are estimated by fitting the general solution obtained for O(t) with the data of cumulative APM equivalent observed in urine as metabolites by Feldmann and Maibach (1974), after an iv dose.

Figure 3 illustrates the very good fit of the predicted O(t) as compared to the observed data. The values of elimination rate constants that provide the best fit are:

k_{o} = 0.0162 hr^{-1} \quad k_{elim} = 0.0213 hr^{-1} \quad k_{n} = k_{elim} - k_{o} = 0.005 hr^{-1}

The corresponding half-life of body burden elimination (T_{1/2,elim}) is:

T_{1/2,elim} = 32.6 hr

The ratio k_{o}/k_{elim} = 0.761, means that 76.1% of the total body burden is eventually eliminated through urine after a long time period (in practice, more than ten days), as the observed metabolite level, k_{o}/k_{elim} = 0.239 represents the proportion of the total body burden eliminated by non-observed pathways. This is consistent with the observation of Feldmann and Maibach (1974) that 69.5% is eliminated as urinary metabolites after 5 days. Our model predicts 69% after 5 days (see Fig. 3).

**Second Step: Determination of the Transfer Rate Constant k_{abs,31} and the Fraction of the Dermal Dose that Was Absorbed f_{abs}**. For a single dermal dose, the general solution for O(t) depends on the dose, on k_{abs,31} and k_{o}, but also on the transfer rate constant of APM from skin to blood k_{abs,31} and the fraction of the exposure that is bioavailable f_{abs}. The latter two parameters are estimated by fitting the general solution of the model for O(t) with the data of the cumulative percentage of APM equivalent in urine observed by Feldmann and Maibach (1974) after a single dermal dose applied on the forearms of volunteers. For this fit, the elimination rate constants k_{elim} and k_{n} used are those obtained in the first step as they pertain to internal body mechanisms that apply, once the dose has been absorbed by whatever means.

Again, Fig. 4 illustrates the very good fit of the simulation to the observed data. The value of the transfer rate constant that provides the best fit is: k_{abs,31} = 0.1561 hr^{-1}, and its corresponding half-life is T_{1/2,elim} = 4.4 hr. Under the conditions of dermal exposure of that experience, the best fit for the fraction of the total dose absorbed is: f_{abs} = 16.1%, that is to say 16.1% of the administered dose was absorbed by the skin.

**RESULTS**

**Inferences from the Proposed Model**

Once the parameters relevant to absorption, elimination, and observation are determined from these controlled experiments, the model allows us to make inferences about many situations of interest. Typical situations are presented next to illustrate...
that it is possible to reconstruct the history of the APM body burden and that of the dose absorbed as a function of time, from the urinary excretions after exposure.

**One-day exposure.** Consider a person who has absorbed, through the skin, 1 unit/kg bw as the result of 5 consecutive hours of exposure (absorption of 0.2 unit/kg bw/h × 5 h). Here the unit of dose may be any amount. From the results of the simulation, Fig. 5 shows the fraction of the total absorbed dose present in the body at any time, for the 20 days following the beginning of exposure. The maximum, in this instance, is reached towards 17 h after the beginning of exposure. At that time, the APM body burden equals 72.8% of the total absorbed dose.

Figure 6 shows the fraction of the total absorbed dose excreted per h as urinary metabolites, for 20 days. The cumulative urinary excretion for 20 days represents 76.1% of the total absorbed dose, i.e. the maximum amount (k0/k_elim) that may be excreted as the observed metabolites in urine. Table 2 shows how the model can predict the expected excretion for each day from the beginning of exposure. Thus, the total dose absorbed can be inferred, knowing the date of exposure and any 24-h post-exposure urinary excretion level of metabolites.

**Several consecutive days of exposure.** Similarly, the body burden of APM as a function of time, and the corresponding doses, can be inferred from the urinary excretion per unit of time in the situation of repeated daily exposures. Consider a daily dermal absorption of 1 unit/kg bw as the result of a 5-h uniform exposure (absorption of 0.2 unit/kg bw/h × 5 h) for 9 consecutive days.

Figure 7 shows the result of the simulation for the fraction of the absorbed daily dose present in the body at any time, for 20 days from the beginning of exposure. We see that a near steady state level is reached around the 9th day, when a body burden level of 208% of the absorbed daily dose is reached. This is nearly 3 times the maximum value reached after a single day’s exposure of the same type.

Figure 8 shows the percentage of the APM daily dose that is excreted as urinary metabolites per h, for 20 days. As expected, the maximum urinary excretion/h at steady state is approximately 3 times the maximum rate obtained after a one-day

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**FIG. 4.** Cumulative percent of APM dose excreted in urine as AP metabolites, as a function of time, following the application of a dermal dose. Data represents the mean of observed data in the study of Feldmann and Maibach (1974) for 6 volunteers. Dotted line represents the predicted cumulative excretion, using the kinetic model and a best fit for k_abs,bl and f_abs.

**FIG. 5.** Simulation of the percent of the total absorbed dose contained in the body at any time from the beginning of exposure, from a single continuous and uniform five h of exposure.
exposure, since excretion/h is deemed proportional to instantaneous body burden.

Note that at steady state, equilibrium is attained between the daily absorbed dose and the total amount of APM excreted each day as either urinary metabolites or non-observed excretions. For instance, when a person absorbs 0.1 mg of APM/kg bw/day (0.35 μmole/kg bw/day) for more than 9 consecutive days, she or he excretes the same amount per day. Since only 76.1% of the total excretion is via the urine metabolites, the daily amount of metabolites found in urine will be 0.761 × 0.35 μmole/kg bw/day = 0.266 μmole/kg bw/day.

**Determination of the NOAEL**

After analysis of the literature on actual human exposures to APM, we believe that the NOAEL (no observed adverse effect level) may be determined from the monitoring of the urinary alkylphosphate metabolites—dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethylidithiophosphate (DMDTP).

The results of the McCurdy *et al.* (1994) study on the reduction of RBC-AChE activity and its link with excretion of the three AP metabolites, DMP, DMTP, DMDTP, together with the parameters of the proposed model between dose, body burden and urinary metabolites, provide the basis for the NOAEL. The parameters were determined from the data of Feldmann and Maibach (1974) who measured total urinary metabolites as 14C excretion and not as specific APs. The three AP metabolites DMP, DMTP, DMDTP are the primary dialkyl-phosphate urinary metabolites resulting from APM exposure, since the P=S and P-S-C bonds of APM produce these three dimethylated alkylphosphates metabolites (Coye *et al.*, 1986c; Drevenkar, 1991). To our knowledge, neither intact APM nor other metabolites of APM have been measured in urine, so it is reasonable to assume that the 14C excretions measured in urine by Feldmann and Maibach (1974) are largely made up of those three AP metabolites.

Here is a brief description of the McCurdy *et al.* study: Twenty farm workers were exposed for 21 days, distributed over 6 weeks (on average for 7 ha day), in peach orchards where APM had been applied 30 days before. No more pesticide applications were made during the time of the study. Twelve subjects who did not perform agricultural work served as controls for the study. None of the participants reported pesticide use or exposure in the 2 weeks preceding field entrance. The urinary AP metabolites measured were DMP, DMTP and DMDTP. They were collected 6 days before the exposure began and on days 1, 2, and 3 when the work began, as well as on the last day of work in the orchards (day 44).

In order to study the effect of APM on cholinesterase enzymes, plasma-ChE/RBC-AChE activity and oxime reactivation were measured in blood samples collected from subjects 6 days prior to the exposure and on day 3 of exposure. Eleven

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**TABLE 2**

Single Day Exposure, Predicted Fraction of the APM Absorbed Dose Excreted As Metabolites (AP) Since Exposure Began

<table>
<thead>
<tr>
<th>Day</th>
<th>Dose % excreted/day</th>
<th>Cumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.9</td>
<td>20.9</td>
</tr>
<tr>
<td>2</td>
<td>21.9</td>
<td>42.8</td>
</tr>
<tr>
<td>3</td>
<td>13.4</td>
<td>56.2</td>
</tr>
<tr>
<td>4</td>
<td>8.0</td>
<td>64.2</td>
</tr>
<tr>
<td>5</td>
<td>4.8</td>
<td>69.0</td>
</tr>
<tr>
<td>6</td>
<td>2.9</td>
<td>71.9</td>
</tr>
<tr>
<td>7</td>
<td>1.7</td>
<td>73.6</td>
</tr>
<tr>
<td>8</td>
<td>1.1</td>
<td>74.7</td>
</tr>
<tr>
<td>9</td>
<td>0.6</td>
<td>75.3</td>
</tr>
<tr>
<td>10</td>
<td>0.4</td>
<td>75.7</td>
</tr>
</tbody>
</table>
subjects had blood drawn on day 44 of exposure for similar measures. The oxime reactivation reaction offers a method for evaluating exposure in the absence of baseline values of plasma ChE/RBC-AChE activity. When an oxime reactivator is given soon after exposure to OPs, the increase of plasma-ChE/RBC-AChE activity is an index of exposure to OPs.

Median decreases for RBC-AChE of 7% on day 3 and 19% on day 44 were observed. The highest observed decrease in RBC-AChE activity in one person was around 25%, and none of the workers showed significant oxime reactivation. If we consider that a 30% depression in RBC-AChE activity (Coye et al., 1986a and b; Richter et al., 1992) as the level where clinical symptoms in more susceptible humans may become observable, we can say that in the McCurdy et al. study, we are in the presence of a NOAEL. However, a decrease of 10% or more in RBC-AChE activity is usually considered a biologically significant effect, even with no clinical symptoms (WHO, 1987).

Again according to McCurdy et al. (1997), the exposure had decreased after the first 3 days. When exposed to a constant daily dose for consecutive days, the model predicts that after 3 days, a body burden of 158% of the absorbed daily dose is reached and, after a prolonged exposure, a maximum of 208% may be reached. Using our model and computer simulations (assuming a mean body weight of participants of 70 kg), we were able to show that for the first three days, the median amount of AP excreted in urine per day was indicative of an average absorbed dose of 0.054 mg APM/kg bw/day. For the most exposed person in that study, we estimated an absorbed dose equivalent to 0.122 mg APM/kg bw/day. This suggests that an absorbed dose of 0.1 mg/kg bw/day is a safe NOAEL for adults.

With an absorbed dose of 0.1 mg/kg bw/day through chronic exposure, a steady state is reached with a body burden equal to 0.208 mg/kg bw; this corresponds, for a person of 70 kg of weight, to a maximum body burden of 14.9 mg. At steady state,
the total amount of urinary AP excreted/24 h is 18.7 μmoles, or 0.266 μmoles/kg bw/24 h.

In contrast, to reach a similar body burden after a single one-day exposure, a person would need to have absorbed a dose nearly 3 times higher (208%/72.8%) or 0.3 mg/kg bw. Such a person would excrete an amount of AP metabolites on the first and second days from the beginning of exposure of respectively 0.215 μmoles/kg bw and 0.225 μmoles/kg bw. For a person of 70 kg, these values correspond to 15.05 μmoles the first 24 h and 15.75 the second 24-h post-exposure.

**Proposed Test**

Based on our results, a biomedical test can be designed to assess the amount of APM absorbed by a subject and a maximum tolerable dose corresponding to our proposed NOAEL. We suggest:

1. From a single one-day exposure: 0.215 μmoles/kg bw/24 h as the tolerable level of urinary AP metabolites for the first or second day from the beginning of exposure. Alternatively, if only data for subsequent days is available, Table 2 can be used to estimate the APM total dose absorbed and to establish whether the NOAEL body burden was reached.

2. For workers exposed repeatedly for more than 5 days: 0.266 μmoles/kg bw/24 h as the tolerable level of AP metabolites measured in urine, for each day of exposure.

Since our results are based on rates of metabolite excretion, they are only dependant on maximum body burden and associated absorbed doses, without regard to the fraction of absorption.

**DISCUSSION**

We have shown that a simple kinetic model for the absorption and the elimination of APM yields time-dependent functional links between dose, body burden, and excretion rates. However, such a model contains free parameters (absorption and elimination rate constants and the bioavailable fraction) that have to be determined from controlled experiments. Fortunately, the iv and dermal-exposure experiments of Feldmann and Maibach (1974) provide the data to estimate these parameters, as described before. From this basis, it is possible to infer the past history of the APM body burden and of the skin-absorbed dose from metabolite excretion data.

To establish the NOAEL, we have used the above results in conjunction with the data of McCurdy et al. (1994) who measured RBC-AChE activity as a function of time together with urinary metabolites (AP) in orchard workers exposed to APM. All of the workers showed reductions in RBC-AChE activity, in some cases by as much of 25%, but none showed any observable adverse effects. Since ill effects were observed at the level of 30% depression in RBC-AChE activity (Coye et al., 1986a and b; Richter et al., 1992; Sidell, 1994), we consider that the levels reached by the orchard workers (McCurdy et al., 1994) should be used as NOAEL.

Starting from the observed metabolite excretion rates, the kinetic model allows us to estimate the maximum body burden attained and the absorbed doses associated with these levels of reduced RBC-AChE activity. For repeated daily exposures, this leads us to propose a NOAEL absorbed dose of 0.1 mg APM/kg bw/day, which at steady state (after approximately 9 days) would result in an excretion of AP metabolites of 0.266 μmol/kg bw/day. On the other hand, for a single, one-day exposure, the proposed NOAEL absorbed dose is 0.3 mg APM/kg bw, which would result in an excretion of AP metabolites on the first and second day from the beginning of exposure of respectively 0.215 μmoles/kg bw and 0.225 μmoles/kg bw. The 3-fold factor between the NOAEL-absorbed dose for a single exposure versus repeated daily exposures stems from the kinetic model’s prediction that the peak body burden, after a single-day exposure, is 72.8% of the dose, as compared to a plateau of 208% of the daily dose for repeated exposures. The interplay between the skin absorption rate and the elimination rate determines the maximum body burden; it yields a different outcome under a steady-state regime when compared to a single day’s dose.

Thus, both NOAEL correspond to a similar maximum body burden. Of course, for chronic exposure, the relation between body burden and RBC-AChE is complex. Indeed, while every day of exposure a fraction of RBC-AChE is inhibited by absorbed OPs, a fraction of old erythrocytes (RBC) that contains inhibited RBC-AChE is removed by the liver (about 1/120, since 120 days is the average lifetime of RBCs). Furthermore, there exists a tolerance effect with time, at the synapse level, for a given inhibition of AChE. By using the same maximum body burden as the underlying criteria for the NOAEL for either repeated exposures or single exposure, without folding in possible tolerance effects, we are on the safe side from a public health point of view.

It is important to note that the above doses refer to actually absorbed doses and not to exposure doses whose fractional bioavailability may vary considerably from one exposure situation to another. Our proposed values for NOAEL doses are thus independent of particular exposure context, as long as absorption is through the skin.

The above figures proposed for NOAEL are consistent with the findings of other researchers. Aprea et al. (1994) have measured the same three metabolites (APs) as biomarkers of APM exposure in 11 orchard workers. They measured these metabolites as μmol/g creatinine. The worst case was a subject working without gloves who showed an average elimination of 3.990 μmol/g creatinine. On the basis of Alessio et al. (1985), daily creatine elimination ranges from 0.5 to 3.0 g. Taking this highest figure and assuming a bw of 70 kg, this worker excreted at most an average 0.171 μmol AP/kg bw/day. According to Aprea et al. (1994), none of the workers showed any reduction in RBC-AChE activity. This is compatible with our
proposed NOAEL, as measured through urinary excretion of AP metabolites.

Rider et al. (1967; 1968; 1970; 1971; 1972) conducted several experiments on human volunteers, each experiment using a different dose level. The doses were administered orally every day for thirty days, and no significant reduction in RBC-AChE activity was observed with an oral dose of 0.2 mg APM/kg bw/day. This is twice the figure we suggest for an absorbed NOAEL dose, but most likely only a fraction of a dose administered by Rider was absorbed, and a tolerance effect due to repeated exposure could have been developed in those exposed volunteers. Similar experiments carried out on rats and dogs (Chemagro Division Research Staff, 1974) showed that the dose level at which cholinesterase is not affected is 0.125 mg APM/kg/day.

It is known that there is a genetic variability in cholinesterase activity among individuals of the general population. Coye et al. (1986c) reported the following interindividual variation of RBC-AChE: mean ± SD activity was 0.766 ± 0.081 ΔH units (male) and 0.75 ± 0.082 ΔH units (female). According to Jeyarathan and Maroni (1994), coefficients of variation in RBC-AChE activity among individuals of the general population have been determined to be in the order of 10–18%. These authors note that RBC-AChE shows no difference in activity between sexes when the sex-related difference in red-cell packed volume is taken into consideration. There is no significant variation with age, with the exception of the less-than-6-month-old infants who have values lower than adults.

Human health risk assessment is conducted to define “acceptable” levels of exposure to chemicals that may exist as contaminants in food, drinking water, air, or the environment. NOAEL or LOAEL (lowest observed adverse effect level) are generally used as guidelines to determine the safety of exposure (of course, a reference dose (RfD) or reference concentration (RfC) may be defined to take into account uncertainty and sensitive subgroups).

On the basis of the data of Feldmann and Maibach (1974), we have seen that eventually 76% of the absorbed APM is eliminated in the form of urinary metabolites. Although not all of the APM is eliminated via this route, the latter is a reliable indicator of the body burden that can be collected easily and with sufficient accuracy. When coupled to a reasonable model for the underlying absorption and elimination kinetics, the daily measuring of cumulative urinary metabolites (APs) provides a simple, specific and sensitive test to estimate body burden, infer absorbed doses, and establish danger levels.

The model developed here is heuristic and very general in nature, makes few physiological assumptions, and is based on data that warrant the use of a first order elimination kinetics through observed or unobserved pathways. The model admits of a finite absorption rate from skin to blood, a matter of interest since skin is the most important route of APM absorption for orchard workers (Aprea et al., 1994).

Different OP compounds have different absorption and elimination rate constants; it is the authors’ intention to apply the approach described here to compounds other than APM and to suggest, if required, the appropriate controlled experiments needed to establish the relevant parameters.

REFERENCES


