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Biodisposition Study of the Organophosphorus Pesticide, Methyl-Parathion

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The biological monitoring of pesticide residues and metabolites is important in the surveillance of occupationally and environmentally exposed individuals. Detection of these compounds in the body indicates that an exposure has occurred, that the pesticide is bioavailable, having been absorbed, and that a dose to critical tissues may have been incurred (Nauman et al., 1994).

In the case of organophosphorous pesticides there is no evidence of prolonged storage in the body. However, several cases of acute intoxication in which symptoms recurred at intervals, especially associated with periods of mobilization from adipose tissue have been reported (Ecobichon, 1977). Therefore, in the therapy of an intoxicated person it must be clear that acetylcholinesterase may be inhibited by the pesticide released from body deposits, long after the bulk of the inhibited enzyme has aged (WHO, 1986) and it may not be possible to distinguish between the toxicant still being absorbed from skin or intestine from that stored very early in the illness and later mobilized from fat (Svetlicic, 1960).

Although methyl-parathion (O-O-dimethyl-O-(4-nitrophenyl) phosphorothioic ester) is one of the organophosphorus insecticides most frequently used in Spanish agriculture, and sometimes has been involved in human poisoning cases (García-Repetto, 1995c) there is not complete information in the literature about its disposition and persistence in the body. Consequently, we performed the present study in which we not only studied its concentration profile in blood but also in tissues (adipose tissue, brain, muscle and liver) in order to see if any accumulation occurs and if any redistribution process exists.

MATERIALS AND METHODS

Methyl-parathion was supplied by Ehrenstorfer's Labora-

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tory (Ausburg, Germany). Male albino Wistar rats (IFA-CREDO, Lyon, France) were used and kept in animal housing in accordance with Good Laboratory Practice. Rats' weight ranged between 200-250 g at the beginning of the experiment.

21 animals were grouped in lots of 3. l/3 of the oral LD_{50} of methyl-parathion (3 mg/Kg) was given by gavage to each animal, while fasting, using olive oil as vehicle (solutions of 0.15 mg/mL of methyl-parathion were used; the volumen adiministered was adjusted to the individual weight of each animal, in order to give them the foremen-tioned doses). Each lot was sacrificed after anaesthesia in an atmosphere of ethylether, once a pre-set time for each group had elapsed (4,8,12,16,20 and 30 days). Blood was extracted by intracardiac puncture and collected in heparinized tubes to avoid clotting. Adipose tissue, muscle, brain and liver were also removed.

Samples (blood, adipose tissue, muscle, brain and liver) were processed according to the method of Zweig(1972) by means of homogenization and cold extraction with methanol, modified in our laboratory by the addition of a step of purification by chromatography through columns of C_{18} (octadecylsiloxane), and elution with n-hexane-:ethylether (1:1). The extracts were evaporated to dryness under a stream of N_2. Dry residues, once redis-solved in methanol, were analysed by gas chromatography using a Perkin-Elmer model 8700 chromatograph with NPD. The 15m x 530µ internal diameter column had methyl silicone as stationary phase. The operating conditions were: injector and detector temperatures 250 and 300° C respectively, carrier gas (He) flows 15 mL/min, oven temperature 150°C for 2 minutes, increasing by 10 °C/min to 250°C, remaining at this temperature for 10 minutes. Internal standards were the aliphatic trialkylamines (Cn=5,Cn=6,Cn=7)(Watts and Simmiek, 1987).

Once the concentrations of pesticide residues in the different tissues were calculated, their evolution with time was observed. PCNONLIN (1985) was the software program used to make the toxicokinetics study of methyl-parathion. PCNONLIN is supplied with a library of the most commonly used pharmacokinetic models and also allows implementation of original models. It is capable of fitting models to data (i.e. finding a mathematical equation and a set of parameters' values such that values predicted by the model are close to the observed val-ues).

To establish the toxicokinetics' profile of methyl-parathion similarities and differences in the behaviour of the pesticide in each tissue studied were observed. The criteria followed to include the tissues in com-partments were: time at which maximum concentration was

reached, half-life, physiological and metabolic characteristics of each tissue and type of mathematical function which best described methyl-parathion's concentration changes with time (García-Repetto, 1995b). The mathematical functions defining those compartments were also calculated by nonlinear regression, using the same software program PCNONLIN.

RESULTS AND DISCUSSION

The detection limit of our analytical method for the pesticide studied was calculated as 3 times the chromatographic noise level (Chamberlain, 1985); being 0.13 µg/ml. The average percent recoveries of methyl-parathion (70.66%- 82.88%) from tissues, which were spiked with a known quantity, are shown in Table 1, being the coefficient of variation values between 0.29 and 5.68% which are considered satisfactory for p.p.m. level determination of organophosphorous pesticides. The method produced linear curves for the spiked samples (average of six replicates) in the range 0.1-0.01 µg/mL whose regression equations (y= 32.28x-0.036) yielded a correlation coefficient of 0.9997. Table 2 shows the changes in concentration (ppm) with time for methyl-parathion in various tissues. Consequently, we consider our analytical method sensitive, linear and with good recovery to perform the toxicokinetic study (DFG, 1992).

First of all, using these concentration/time pairs of data the mathematical functions that best described concentration evolution with time in each tissue were estimated. The data obtained for all the tissues fitted the following equation:

$$-C_{(t)} = C_0 e^{kt} - C_1 e^{-K't}$$

The calculated half-life ($t_{1/2}$) of methyl-parathion from k' (elimination constant):

$$-t_{1/2} = \text{Ln } 2/K'$$

Table 3 shows these equations for each tissue with their correlation coefficients (r values higher than 0.8 are acceptable for non linear regression) (Myers, 1990) and also the half-life ($t_{1/2}$) of methyl-parathion in each tissue.

Secondly, based on the described functions tissues were grouped in compartments according to the previously mentioned criteria defined by compartmental equations.

Tissues studied were singled out for the following reasons:
- Total blood instead of serum or plasma because methyl-parathion is a lipophilic substance (Merck, 1989) which

may be partitioned to red cells.

- Brain because it is the target organ for these kinds of compounds and data obtained from it may be more closely associated with adverse effects and thus useful in estimating health risk .

- Adipose tissue and muscle as indicators of whether methyl-parathion accumulates.

- Liver because it is the organ where methyl-parathion's metabolism occurs principally (Dauterman, 1971).

Sampling was programmed in order to demonstrate accumulation and post-distribution processes whenever they occurred. Sampling is not as crucial for those pesticides which are stored in adipose tissue and are released slowly but continously to the blood, as it is for those pesticides that are rapidly eliminated from the blood (Nauman et al., 1994). This experiment, as it was designed, may increase the existing knowledge about methyl-parathion biodisposition and also be useful in understanding the complete clinical picture of the poisoning, including the duration of the illness.

Observation of the results obtained in the study of methyl-parathion (Table 2) shows that in each tissue it presented a delayed peak of concentration (8 and 12 days respectively). From the maximum, the elimination of the pesticide is exponential in all tissues. Actually, the fact of a delayed excretion of an organophosphorus compound is not due to slow metabolism as it happens with organochlorine pesticides. It is due to storage in certain tissues which maintain the compound unavailable for metabolism (Hayes, 1991).

Tissues were grouped in compartments according to the criteria described above. Blood and liver were included in one compartment due to a similar concentration profile, reaching maximun methyl-parathion's level 12 days after oral administration with a half-life in both tissues of approximately 15 days. Elimination is believed to take place always from this compartment, which is called central compartment and is represented by the following equation: $C=2.43e^{-0.22t}-49.09e^{-0.26t}$ whose correlation coefficient with data is 0.999. This compartment is in accordance with the first pass effect described by Braeckman et a1.(1983) for methyl-parathion in the dog.

Brain and adipose tissue made up two independent compartments, in spite of presenting similar evolution of concentrations with the maximun at 8 days. It was impossible to find a mathematical function which could define a joint compartment properly. When nonlinear

Table 1. Average recovery percent of methyl-parathion from tissues[a]

Pesticide	Blood	Adipose Tissue	Muscle	Brain	Liver
Methyl-Parathion	79.61% ±1.57 1.97%	82.88% ±9.04 11.17%	70.66% ±0.39 0.55%	71.91% ±2.76 3.84%	81.33% ±3.55 4.70%

[a] Table 1 shows the average recovery percent, its standard deviation and the coefficient of variation for each determination (3 trials).

Table 2. Methyl-parathion's concentration profile along with standard deviation and variation coefficients[a].

Time (Days)	Blood	Adipose Tissue	Muscle	Liver	Brain
4 Hours	N.D.	N.D.	N.D.	N.D.	N.D.
4	1.09 ±0.014 1.34%	0.87 ±0.023 2.64%	N.D.	0.08 ±0.0026 3.25%	0.32 ±0.0051 1.59%
8	1.54 ±0.018 1.16%	1.53 ±0.085 5.55%	N.D.	0.145 ±0.0021 1.45%	0.4 ±0.014 3.50%
12	2.26 ±0.072 3.18%	0.47 ±0	N.D.	0.18 ±0.0014 0.77%	0.28 ±0
16	0.59 ±0.013 2.20%	0.41 ±0.0051 1.24%	N.D.	0.055 ±0.0008 1.45%	0.21 ±0.008 3.81%
20	0.43 ±0.014 3.25%	0.35 ±0.006 1.71%	N.D.	0.046 ±0.0013 2.83%	0.13 ±0.0048 3.69%
30	N.D.	N.D.	N.D.	N.D.	N.D.

[a] N.D. means not detected.

Table 3. Regression equations and half-life of methyl-parathion in each tissue studied.

TISSUE	REGRESSION EQUATION	r	$t_{½}$ (DAYS)
Blood	$C= 0.47e^{0.13t}-50.73e^{-0.26t}$	0.999	14.67-±0.14
Adipose Tissue	$C= 0.27e^{0.21t}-4.34e^{0.14t}$	0.994	13.01 ±0.46
Liver	$C= 0.04e^{0.13t}-2.96e^{-0.23t}$	0.984	14.95 ±0.65
Brain	$C= 0.1e^{0.17t}-0.90e^{-0.10t}$	0.985	14.91 ±1.06

regression was applied to these concentration/time pairs of data a wrong range matrix of partial derivatives was reached (Myers, 1990). This pesticide was not detected in muscle at any time during the study (Table 2).

From the obtained results we can conclude that methyl-parathion is distributed tricompartmentally. Said model is made up of one central and two peripheral compartments (Gibaldi, 1982). In the proposed toxicokinetic model we accepted that once methyl-parathion's distribution to tissues is completed, a redistribution process from tissues to blood begins. This process means a release of methyl-parathion molecules which may interact with acetylcholinesterase (WHO,1986) long after the bulk of the inhibited enzyme has aged and cause new symptoms which are known as an endogenous reintoxication if the quantity released is sufficiently high.

Concentration levels of methyl-parathion in blood are higher than those found in tissues, showing certain preference for adipose tissue. Although levels of methyl-parathion in adipose tissue were higher than those of the other tissues, they were too small in comparison to blood levels to conclude that they acted as a deposit compartment where accumulation takes place and thus that the quantity of methyl-parathion released to the blood would cause an endogenous reintoxication (García-Repetto et al., 1995a). In the same way it seems improbable that the low quantities of methyl-parathion mobilized from other tissues to the blood may cause new symptoms. However, on the whole, the results suggest the need for further study in order to confirm this hypothesis.

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