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Dermal Penetration Rate and Pharmacokinetics of the Insecticide Methidathion in Sprague-Dawley Rats

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ABSTRACT *: The skin penetration rate of methidathion in vitro and pharmacokinetics of methidathion in vivo were studied with male Sprague-Dawley rats by dermal treatment. The in vitro skin penetration rates for Sprague-Dawley rats of methidathion technical (50 mg, 100 μl) and emulsifiable concentrate (EC, 40 mg, 100 μl) were determined as 18.4 μg/cm^2/h (RSD = 6.5) and 18.5 μg/cm^2/h (RSD = 3.2), respectively. Dose-related systemic exposure (AUC) was observed in rats after dermal treatment. The corresponding AUC, T_{max}, C_{max}, and $T_{1/2}$ of methidathion in plasma were 1.5 μg· hr/ml, 6 h, 0.10 μg/ml, and 16 h, for 116 mg/kg doses, 3.2 μg· hr/ml, 8 h, 0.12 μg/ml, and 23 h, for 232 mg/kg doses and 10 μg· hr/ml, 12 h, 0.32 μg/ml, and 20 h, for 1,160 mg/kg doses respectively. The urinary excretion of methidathion, estimated using an equation derived from the in vitro skin penetration study was 0.24~0.35% of the absorbed dose. The concentration of methidathion in kidney was higher than that in liver. Dose-dependent absorption and excretion of methidathion without saturation was observed under in vivo experimental condition.*

Key Words *: Methidathion, Penetration rate, Skin, Blood, Excretion*

I. INTRODUCTION

Human exposure to pesticides can occur during manufacture, mixing/loading, spraying, harvest, and by consumption of treated crops. By far the greatest potential exposure is through the dermal absorption during spraying operations, and the importance of related exposure has been confirmed by many studies (Durham *et al.*, 1972; Ecobichon, 1996; Feldman and Maibach, 1974; Fenske and Elkner, 1990; Turnbull *et al.*, 1985; Wolfe *et al.*, 1996). Therefore, measurement of dermal absorption, resulting distribution in blood and tissues, and urinary excretion are considened to be important for the safety evaluation to understand the potential in-use hazards.

Methidathion [S-2,3-dihydro-5-methoxy-2-oxo-1,3,4-thiadiazol-3-ylmethyl *O,O*-dimethyl phosphorodithioate] is an organophosphorous insecticide which has been used for the control of a wide range of sucking and chewing insects and spider mites on fruits and vegetables in Korea (Korean Pesticide Industry Association, 2000). It is poorly soluble in water (solubility: 200 mg/l), but is rapidly hydrolyzed in alkaline and strongly acidic media (Tomlin, 2000). The main routes of its metabolism in animals and plants are hydrolysis, oxidation and methylation (Chopade *et al.*, 1981; Dupuis *et al.*, 1971; Roberts and Hutson, 1999).

The reliable analysis of methidathion in biological samples is a critical step in many studies and was carried out by gas chromatography (GC) or high performance liquid chromatography (HPLC) with a variety of detectors (Balint *et al.*, 1995; Cho *et al.*, 1997; Holstege *et al.*, 1994; Kawasaki *et al.*, 1992; Liu *et al.*, 1989; Sharma *et al.*, 1990; Szegletes *et al.*, 1995; Tsatsakis *et al.*, 1996; Ueda *et al.*, 1993).

Recently, dermal exposure of methidathion was evaluated in greenhouse workers (Kim *et al.*, 2000) however, no information was available on its dermal absorption, distribution and excretion, which are the important factors for the proper exposure risk assessment.

In the present study the skin penetration rate of methidathion was studied *in vitro* with glass diffusion

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cell, using HPLC analysis. The *in vivo* pharmacokinetics of methidathion was further investigated to elucidate its fate *via* skin absorption with a validated analytical method using gas chromatography/flame photometric detector (GC/FPD).

II. MATERIALS AND METHODS

1. Chemicals

Methidathion technical (96.2%) and EC (40% active ingredient) were kindly provided by Syngenta Korea (Seoul, Korea). HPLC grade acetone, hexane, ethyl acetate and methanol were purchased from Duksan Co. (Ansan, Korea). All other chemicals and reagents were analytical grade and commercially available.

2. Animals

Male Sprague-Dawley (SD) rats, 5 weeks old, were received from SamTako Bio Korea (Osan, Korea) and were held in the animal cage for at least 1 week to acclimate prior to experimental use. Rats were housed under specific pathogen-free conditions at 23±1°C and a relative humidity 50±5% on a constant 12 h light/dark cycle. One day before the experiment the animals were individually kept in polycarbonate metabolic cages (DaeJong, Seoul, Korea), which are suitable for the collection of urine and feces. The animals (210~250 g), approximately 6 weeks of age at the start of each study, were given sterilized tap water and a certified standard laboratory rodent diet (SamTako Bio Korea) *ad libitum* throughout the experiments.

3. Instruments and method validation

An HPLC system (Hewlett Packard series 1100) consisting of a variable wavelength detector, a Zorbax SB-C18 column (4.6 mm i.d. ×250 mm; particle size: 5 μm, Hewlett Packard) and a guard column (Novapak C_{18}, Waters) was employed for the quantitation of methidathion. The mobile phase was a mixture of methanol and water (70 : 30). The flow rate of the mobile phase was set at 1.0 ml/min and detection was performed at 230 nm

The GC system consisted of Model 5890 II plus (Hewlett-Packard, Avondale, PA, USA), an HP-5 column (30 m×0.53 mm; film thickness: 1.5 mm) and a flame photometric detector (FPD, operated in the P mode). Injector, column oven and detector temperatures were 240, 210 and 260°C, respectively, and carrier gas (N_2) flow rate was 20 ml/min.

The validation of the analytical method was performed according to the suggestions proposed by Bressolle *et al.* (1996). Intercept, slope and coefficient of correlation (r) were evaluated for five calibration curves. The calibration was accepted if the (r) value found was above at least 0.995 (Tsai *et al.*, 2001). Extraction efficiency of methidathion from plasma, urine, liver and kidney was examined using hexane, or ethyl acetate or dichloromethane. Acceptance criteria of the results were based on accuracy values, the percentage ratio between the mean concentration obtained and the nominal value, in the range from 85 to 115%. Precision values were < 20% for low values and < 15% for medium and high values.

4. *In vitro* dermal penetration

The rats were sacrificed after diethyl ether inhalation. The hair of the abdominal region was removed with an electric clipper and the abdominal skin freed of subcutaneous tissue was stripped with scissors. A Teflon O-ring was attached to the excised rat skin on the epidermal side with an adhesive agent. Skin membranes (3.14 cm^2) were mounted in the glass diffusion cell (Tsuruta, 1982) and then the lower chamber was filled with physiological saline (18 ml).

After the cell was pre-incubated in a shaking incubator at 32°C for 2 h, methidathion technical in acetone was treated on the skin surface in various application amounts (20~200 mg) and volumes (50~500 μl), while methidathion EC was applied at two different doses. During the further incubation for 48 h with shaking, the receptor fluid in glass cell was sampled (0.5 ml) at regular intervals. An equal volume of fresh receptor fluid was then returned to the receptor fluid to ensure a constant volume. Samples were stored at -20°C until analyzed by HPLC.

5. *In vivo* dermal pharmacokinetics

Hair was clipped from the intrascapular area on the

backs of rats and each rat was inspected to make sure the skin had not been nicked. Methidathion technical in acetone (200 ml) was applied to a 6.25 cm^2 (2.5×2.5 cm) area of skin at dosages of 116, 232 and 1160 mg/kg (3 rats per each dose level and time point, total of 63 rats). After dosing, the entire area was covered with a gauze (occlusive mode), and secured with wide adhesive tape.

Rats were anesthetized by diethyl ether inhalation at 2, 4, 6, 8, 12, 24 and 48 h after treatment. Blood was sampled from abdominal vein by syringe and urine was sampled from the metabolism cage. The liver and kidney were quickly excised, rinsed with ice-cold saline solution, blotted dry, weighed, frozen, and stored at ≤ -20°C until analyzed. Each urine volume was recorded.

After blood samples were centrifuged (3,000 g, 15 min, 4°C) the plasma (0.5 ml) was acidified with 6 N HCl (0.1 ml), extracted with hexane (0.5 ml) by vortexing (1 min) and centrifuged (10,000 g, 10 min, 4°C). Urine sample (0.5 ml) was extracted with hexane in the same manner as for blood. Each aliquot (5 μl) of hexane phase was analyzed by GC.

Liver and kidney samples were homogenized with a tissue homogenizer (Ultra Turrax T25 basic, Ika Lab., Germany). Homogenate (0.4 g) was mixed with water (0.4 ml), extracted with hexane (0.8 ml) by vortexing (1 min) and ultrasonication (30 min), and centrifuged (10,000 g, 10 min, 4°C). The organic phase was concentrated by purging with a stream of nitrogen gas. The residue was reconstituted with hexane (80 μl) and analyzed by GC. Concentrations of compounds from all samples were determined as the mean of triplicate samples.

6. Calculation and statistical analysis

Pharmacokinetic calculations were performed for average values of each individual data. The maximum peak concentration (C_{max}) and the time to maximum peak concentration (T_{max}) for methidathion were determined by visual inspection from plasma concentration-time curve. The area under the curve ($AUC_{0\sim48\,h}$) was calculated by the linear trapezoidal rule (Martin, 1986) from 0 to 48 hours. Terminal half-life ($T_{1/2}$) was derived by linear regression from the descending part of the log concentration-time curve. Statistical analyses were performed using Student's *t*-test (JMP Statistical Software, SAS Institute Inc., Cary, NC, USA).

III. RESULTS

1. Method validation

A simple direct injection of the receptor solution of diffusion cell resulted in a good HPLC chromatograms without impurity peaks. The linear range of the calibration curve for methidathion was 0.1~100 ppm and the correlation coefficient was 0.999.

GC/FPD also gave a good separation of methidathion from relatively complex biological samples. Hexane extracts contained fewer impurities than the other solvent extracts. The limit of detection for methidathion was 0.01, 0.01, 0.002 and 0.002 ppm for blood, urine, liver and kidney samples, respectively, with a signal to noise ratio of 3, indicating sufficiently low level of detection was achieved for routine analysis. The correlation of peak areas and the concentrations of methidathion was linear over the range of 0.02~2 ppm ($r^2 > 0.995$). The relative standard deviations, which represent the precision of this method, for the slope and correlation coefficient were smaller than 5.7 and 0.1%, respectively ($n = 5$).

The recoveries were reasonable for tissue samples and the accuracy and precision of recoveries were expressed as a mean recovery±standard deviation

Table 1. Mean recoveries of methidathion from blood, urine, liver, and kidney samples

Sample	Recovery (%)[a]				
	0.02 ppm	0.05 ppm	0.1 ppm	0.5 ppm	2.0 ppm
Plasma	NA[b]	88.9±4.8	NA	89.7±2.6	92.3±1.6
Urine	NA	106.0±3.2	NA	96.5±3.1	95.4±5.0
Liver	87.7±6.1	85.3±1.2	90.9±0.8	NA	NA
Kidney	NA	85.6±2.6	90.6±1.6	92.0±3.6	NA

[a]Mean recoveries±standard deviation ($n = 3$).
[b]NA: non applicable.

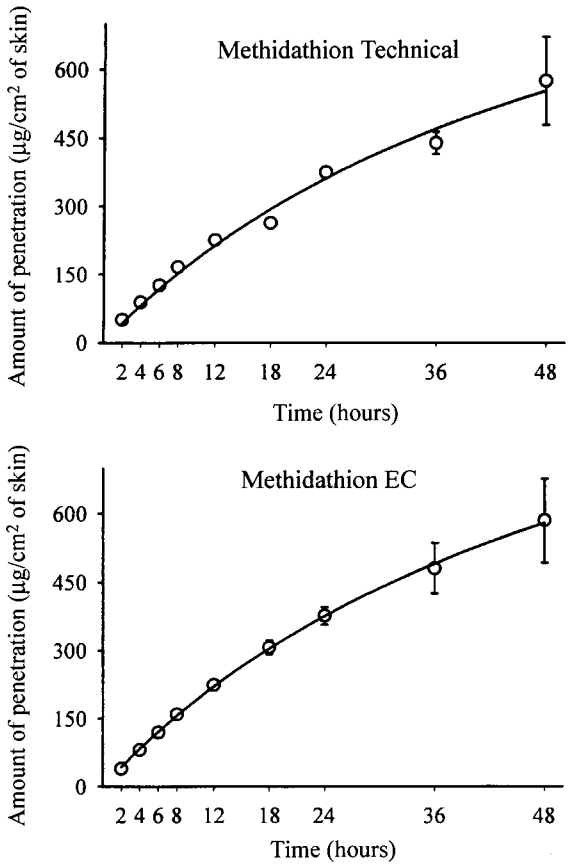

Fig. 1. Penetration curve for methidathion technical (top, 100 mg/100 μl application) and EC (bottom, 40 mg/100 μl application).

(%, n = 3) (Table 1). All %RSD values were < 7%.

2. *In vitro* dermal penetration

The quantity of methidathion that penetrated skin

Table 2. The skin penetration rate of methidathion technical and EC through excised rat skin

Compound	Application amount (mg)	Application volume (μl)	Skin penetration rate[a] (μg/cm²/h)
Methidathion Technical	100	50	12.7±1.4
		100	17.7±0.2
		200	18.1±0.6
		500	17.2±0.9
	20	100	11.5±0.5
	50		18.4±1.2
	100		17.7±0.5
	200		20.0±1.5
Methidathion EC	40	100	18.5±0.6
	100	250	19.5±0.5

[a]Mean values±standard deviation (n = 3).

Table 3. Penetration of methidathion *via* excised skin in 48 h

Total application amount (mg)	20	50	100	200
Application amount/area (mg/cm²)	6.4	15.9	31.9	63.7
Skin penetration rate (mg/cm²/h)	11.5	18.4	17.7	20.0
Total penetration amount (mg)	1.7	2.8	2.7	3.0
Total skin penetration ratio (% of applied dose)	8.5	5.6	2.7	1.5

increased linearly with time for the first 12 hours of incubation for all cases as shown in the plots of the cumulative amount of absorbed methidathion versus the incubation time (Fig. 1). The equation for the steady state linear region (0~12 h) of each graph was obtained by using a method of least-squares and the penetration rate was from the slope of the equation.

When methidathion technical was applied to skin with more than 50 mg in 100 μl of solvent, the skin penetration rate was near to the equilibrium value (Table 2). By applying the skin penetration rate (17.7 μg/cm²/h) at 100 mg and 100 μl level, the amount of penetration was calculated about to be 2.7% of applied dose for 48 h. There was a good correlation (r^2 = 0.982, y = -7.28 log x + 14.2) between application amount (x, mg/cm²) and total skin penetration ratio (y, %) during 48 h (Table 3). However, for methidathion EC, there was no significant difference (p < 0.05) in skin penetration rate between treatment level (Table 2). And no significant difference (p < 0.05) was observed in skin penetration rate between technical and EC.

3. Plasma kinetics

Dose-related systemic exposure (AUC) was observed in rats after dermal treatment (Fig. 2). C_{max}, however, did not increase linearly with increasing dose (Table 4). The pharmacokinetic parameters were obtained by non-compartmental analysis of the concentrationtime curves.

4. Urinary excretion

The mean cumulative curve of methidathion excreted in urine after the dermal treatment increased with time and with dose levels (Fig. 3). When the equation (y = -7.28 log x + 14.2), which was derived from the

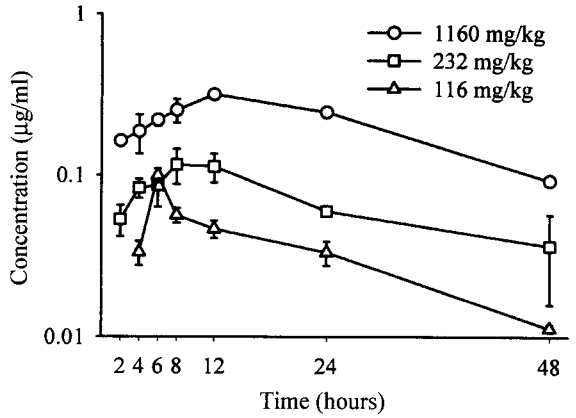

Fig. 2. Mean plasma concentration-time curve of methidathion in male SD rats after the dermal treatment.

Table 4. Pharmacokinetic parameters of methidathion in plasma after the dermal treatment of methidathion to rats (3 rats per each dose level and time point)

Parameter	Dose (mg/kg)		
	116	232	1160
T_{max} (h)	6	8	12
C_{max} (μg/ml)	0.10	0.12	0.32
$AUC_{0\text{-}48h}$ (μg · h/ml)	1.5	3.2	10.0
$T_{1/2}$ (h)	16	23	20

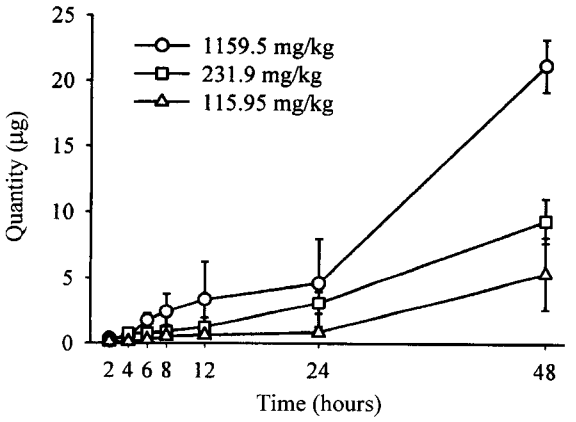

Fig. 3. Mean cumulative quantity-time curve of methidathion excreted in urine after the dermal treatment.

in vitro skin penetration study, was applied to estimated the amount of urinary excretion of non-metabolized methidathion, the small ratio to the absorbed dose (0.24~0.35%) in 48 h was calculated (Table 5).

5. Tissue distribution

In liver and kidney, methidathion was not detected except for the case of the highest treatment level (Table 6), supporting the studies that reported no accumulation of methidathion or its metabolites in any organ of the rat took place (Dupuis *et al.*, 1971). The concentration of methidathion in kidney was higher than that in liver (Fig. 4). The ratio of the concentration in liver to that in plasma (0.02~0.21) was very low (Table 7).

Table 5. Urinary excretion of methidathion after the dermal treatment of methidathion to rats

Total dose (mg/kg)	116	232	1160
Dose/rat (mg/rat)[a]	26.7	53.4	266.8
Dose/area (mg/cm²/rat)	4.3	8.5	42.7
Total excreted amount (μg)	6.4	9.4	21.3
Calculated total skin penetration rate (%)[b]	9.6	7.4	2.3
Total absorbed amount (mg)[b]	2.6	4.0	6.1
% of absorbed dose[b]	0.25	0.24	0.35
% of applied dose[c]	0.02	0.02	0.01

[a]Average weight: 230 g (actual weight 210~250 g)
[b]Results using the in vitro equation (y = -7.28 log x + 14.2).
[c]Total excreted amount/dose per rat.

Table 6. Pharmacokinetic parameters of methidathion in liver and kidney after the dermal treatment of 1160 mg/kg methidathion to rats

Parameter	Tissue	
	Liver	Kidney
T_{max} (h)	8	8
C_{max} (μg/g)	0.06	0.40
$AUC_{0\text{~}48\,h}$ (μg · h/g)	1.2	14.2
$T_{1/2}$ (h)	5	16

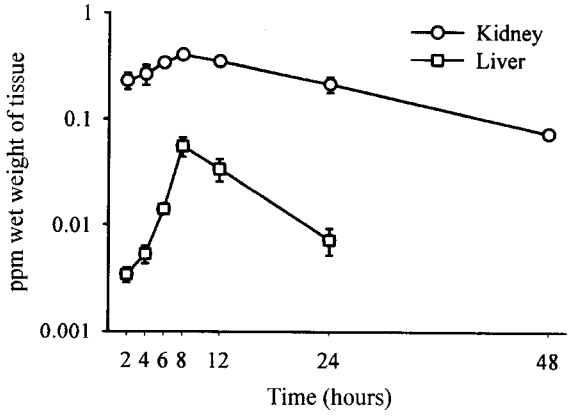

Fig. 4. Mean liver and kidney concentration-time curve of methidathion in male SD rats after dermal treatment of 1160 mg/kg methidathion.

Table 7. Tissue to plasma ratio after the dermal treatment of 1160 mg/kg methidathion to rats

Sample	Tissue to plasma ratio[a]						
	2 h	4 h	6 h	8 h	12 h	24 h	48 h
Liver	0.02±0.00	0.03±0.01	0.07±0.03	0.21±0.06	0.11±0.02	0.04±0.00	-
Kidney	1.4±0.2	1.3±0.5	1.6±0.2	1.6±0.3	1.1±0.0	0.9±0.2	0.8±0.1

[a]Tissue to plasma ratios were calculated for each animal. Data are mean±standard deviation (n = 3).

IV. DISCUSSION

The excised skin has been used in many studies for the measurement of percutaneous absorption of chemicals (Bronaugh and Stewart, 1985; Franz, 1975; Moody, 1997; Tsuruta, 1982). It could reflect the living state (Franz, 1975) and the obtained data correlated well with those obtained from the *in vivo* method (Tsuruta, 1977). In the present percutaneous absorption study of methidathion, the Tsuruta type diffusion cell with excised skin was successfully utilized with aid of HPLC in which the cell fluid was analyzed directly. The results showed no significant difference in skin penetration rate depending on the treated amount or the formulation (Table 2). In 48 h, only a small amount of compound (~2.7%) penetrated skin in case of 100 mg treatment samples.

For the analysis of tissue samples from the *in vivo* dermal pharmacokinetic study of methidathion, GC/FPD was employed after examining of the several related methods. The FPD was more sensitive and selective to phosphorous than other detectors to give 0.05 ng of the minimum detectable quantity with a signal to noise ratio of 3. Hexane was selected as extracting solvent for methidathion in tissue samples because hexane extracts was cleaner than ethyl acetate or dichloromethane extracts. This analytical procedure developed in this study was reliable enough to give the small relative standard deviation.

As shown in Fig. 2 and 3, clear kinetics of methidathion was observed in plasma, liver and kidney after the dermal treatment. The high correlation (r^2 = 0.989) between the dermal dose level of methidathion and the area under the curve ($AUC_{0-48\,h}$) value indicated the dose-dependent absorption of methidathion without saturation under the experimental condition.

The low urinary excretion of methidathion supported the studies that reported only metabolites of methidathion were detected in urine from the rat metabolism study following oral administration (Dupuis *et al.*, 1971) or intraperitoneal injection (Bull *et al.*, 1968). There was significant correlation (r^2 = 0.987) between the amount of recovered methidathion in urine and the dermal dose level, indicating that methidathion was excreted in dose-dependent manner without saturation under the experimental condition.

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