Bull. Environm. Contam. Toxicol. 29, 76–83 (1982)

# Uptake of Insecticides by Intestinal Epithelial Cell Suspensions Isolated from Mice

S. M. Ahdaya and F. E. Guthrie

*Toxicology Program, Department of Entomology, North Carolina State University, Raleigh, NC 27650*

Penetration of insecticides through the gastrointestinal tract appears to be a complex process involving several interacting factors. Previous studies have reported the rate of penetration of insecticides in vitro and in vivo through the intestine (SHAH and GUTHRIE 1970, AHDAYA et al. 1981). These studies showed no consistent correlation between penetration and such physicochemical parameters as partition coefficient and water solubility.

Attempts have been made to isolate the epithelial cell lining of the small intestine from different species of animals utilizing mechanical, chemical and enzymatic techniques and combinations thereof (see review by KIMMICH 1975). Cell suspensions prepared from established cell cultures have been utilized more successfully in a study of cellular uptake than cells isolated from recently killed animals (MURAKAMI and FUKAMI 1976, 1978, 1979).

Due to the inability to identify a single rate-limiting factor for the penetration of insecticides through the intestine (a multi-compartment problem), this study was initiated to examine penetration of selected insecticides in a suspension of epithelial cells.

## MATERIALS AND METHODS

Animals. Seven to eight weeks old, 25-30 gm, female mice, ICR strain, were supplied by Dominion Laboratories, Dublin, VA.

Media. Dulbecco's phosphate buffered saline, $Ca^{++}$ and $Mg^{++}$ free, (PBS) media was purchased from GIBCO Laboratories, Grand Island, NY. Isolating media was composed of PBS plus 240 nM mannitol and 5 mM glucose, 1 mg/ml hyaluronidase, and 10 mg/ml of bovine serum albumin (BSA), pH adjusted to 7.3. Suspending media was composed of PBS-M and 5 mM glucose pH 7.3.

0007-4861/82/0029-0076 $01.60

Enzymes. Hyaluronidase (Type 1) and bovine serum albumin (BSA-fraction V) were purchased from Sigma Chemical Co.

Radioactive insecticides. $^{14}$C-Malathion (succinyl-labeled sp. act. 4.6 mCi/mmol), and $^{14}$C-DDT (U-ring-labeled, sp. act. 29.7 mCi/mmol) were supplied by Amersham Corporation, Arlington Heights, IL. $^{14}$C-Nicotine (pyrrolidine-2-carbon labeled, sp. act. 54.01 mCi/mmol) was supplied by New England Nuclear, Boston, MA., and $^{14}$C-chlorpyrifos (2,6-ring-labeled, sp. act. 12.5 mCi/mmol) was a gift from Dow Chemical Co., Midland, MI.

Epithelial cell preparation. Intestinal epithelial cell suspensions were prepared by a modification of the procedure of KIMMICH (1970). Mice were killed by cervical dislocation, an abdominal incision immediately made, and the small intestine was removed and placed in ice-cold $Ca^{++}$, $Mg^{++}$ free Dulbecco's phosphate-buffered saline plus mannitol (PBS-M). The intestine was flushed and rinsed thoroughly with PBS-M. The preparation was divided into 4 equal segments and everted onto Silastic Medical Grade tubing (Dow Corning, MI). The everted intestine was incubated in the isolated media in a water bath at 37°C. A gentle stream of a mixture of 95% $O_2$-5% $CO_2$ was used during incubation. The intestine was removed after 30 minutes and placed in a test tube containing ice-cold suspension medium to effect minimal proteolytic enzyme activity. It was shaken vigorously for 2 minutes using a vortex mixer. The intestine was removed, and the cell suspension was then centrifuged at 400g for 2 minutes. The supernatant was discarded. The cell pellet was washed two times to remove hyaluronidase. The final cell pellet was suspended in a known volume of PBS-M plus 5 mM glucose. No BSA was used in the final cell suspension.

A few drops of the cell suspension were withdrawn for microscopic examination and determination of cell viability. Epithelial cells were identified by their characteristic columnar shape and by the well-developed brush border (EVANS et al. 1971).

Cell respiration and viability. Respiration was measured by withdrawal of 2 ml samples from the cell suspension every 30 min as described by EVANS et al. (1971). Oxygen uptake was recorded at 37°C with a Clark Oxygen Electrode. Viability was determined by utilizing the trypan blue dye exclusion method.

Uptake of radioactive insecticide. Four µg of each insecticide containing 50,000 dpm was incubated in 2 ml cell suspensions at 37°C in 10 ml Erylenmeyer flasks.

Aliquots (0.2 ml) were withdrawn from the cell suspension and placed in centrifuge tubes containing 2 ml of ice cold suspending medium. The initial aliquot was drawn 5 seconds after mixing the insecticide with the cell suspension, and the subsequent aliquots were withdrawn at 15 second intervals. They were centrifuged at 2000 rpm for 2 min. The cell pellet was washed twice, suspended in scintillation fluid, and counted in a Packard Model 3330 Liquid Scintillation Counter.

Effect of inhibition on uptake of insecticides in cell suspension. Two ml of cell suspension was mixed with 10 µl of 200 µM dinitrophenol (DNP) or 20 mM sodium arsenate. Four micrograms of each insecticide containing 50,000 dpm was added and uptake measured as described above.

## RESULTS AND DISCUSSION

The entire process of isolation from the time of death until preparation of the cell suspension was about 60 min. Microscopic examination from the cell suspension revealed the presence of single epithelial cells, as well as some in aggregate form (Fig. 1). The viability of the isolated cells as measured by trypan blue exclusion was well above 90% for all the preparations made for this study. Treated cells showed appreciable aggregation as compared to control. Lysis was not noted during the uptake observation periods (less than 2 min) but was noted in all treatments within 10 min. Cytotoxicity was not markedly different among compounds (data not shown).

Figure 2 shows the rates of respiration for the isolated cells. The rates were constant for at least 3 hrs and decreased slightly over a 24 hr period. The average rate of oxygen uptake in this study was 0.55 ± .05 µM $O_2$/mg protein/hr. No differences in respiration were noted between treated and untreated cell suspensions. In other studies oxygen consumption was reported to be linear for only a short period of time, less than 30 min (KIMMICH 1970).

All four insecticides studied showed initial rapid uptake (Table 1) but the amounts accumulated differed among compounds. The rate of uptake reached a plateau in 30 to 45 seconds. MURAKAMI and FUKAMI (1976) observed a similar trend with cultured human cells.

Epithelial cells demonstrated a greater ability to accumulate chlorpyrifos and DDT than malathion and nicotine. This could be attributed to the fact that

TABLE 1. The Uptake of Insecticides by Isolated Intestinal Cells

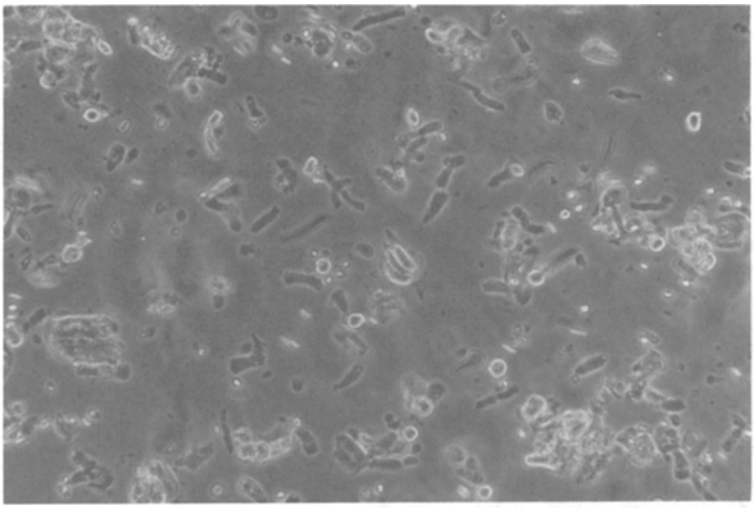
| Insecticide | Time (seconds) 5 | 15 | 30 | 45 | 60 | 75 | 90 |
|---|---|---|---|---|---|---|---|
| Nicotine[a,b] | 2.0±0.1[c] | 2.9±0.4 | 3.4±0.2 | 4.5±0.3 | 4.5±0.3 | 4.2±0.5 | 4.1±0.4 |
| Malathion | 17.5±1.2 | 29.1±2.6 | 34.7±5.1 | 36.6±6.3 | 35.9±1.4 | 38.3±2.9 | 30.4±1.4 |
| Chlorpyrifos | 101.3±6.7 | 152.7±14.9 | 227.5±22.7 | 240.3±21.4 | 253.6±18.3 | 240.3±12.8 | 266.5±23.4 |
| DDT | 69.5±4.3 | 138.5±3.8 | 149.5±2.0 | 152.8±3.8 | 156.6±2.9 | 157.7±4.6 | 160.4±5.4 |

[a]Water Solubility: nicotine, miscible; malathion,145 ppm; chlorpyrifos, 2 ppm; and DDT, 10 ppb (MARTIN and WORTHING 1974).

[b]Partition Coefficient (olive oil; water): nicotine 0.02, malathion 56, chlorpyrifos 1044, and DDT 1775 (SHAH et al. 1981).

[c]ng/mg Protein, ave. of 4 replication and standard deviation.

(a)



(b)

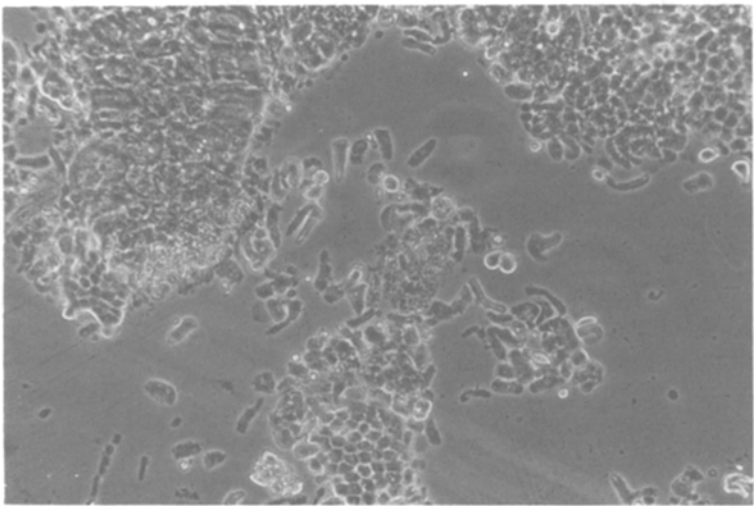


Figure 1. Phase contrast micrographs (310x) of intestinal epithelial cell suspensions of mice taken 10 min after preparation. (a) Control; (b) Chlorpyrifos 2 ppm

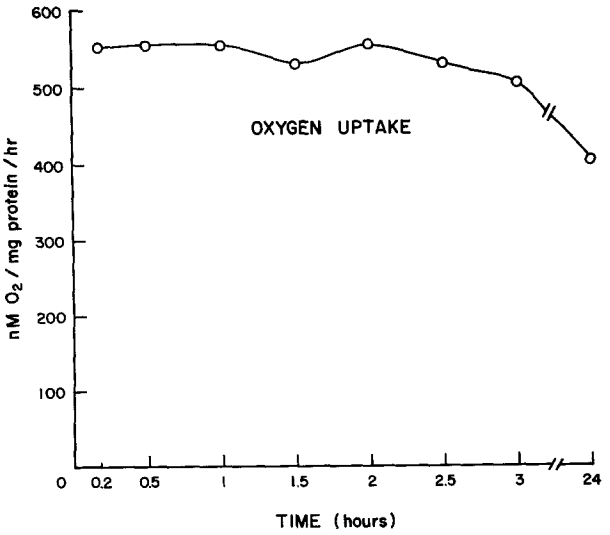



Figure 2. Oxygen uptake rates of untreated cell suspensions over time.

chlorpyrifos and DDT have high partition coefficients while that of malathion is moderate and nicotine low. This is in agreement with the previous findings of MURAKAMI and FUKAMI (1978) using cultured human cells.

In studies using DNP and sodium arsenate as indicators of the energy dependence of the uptake, no effect was seen for DDT, chlorpyrifos and nicotine, while a component of malathion uptake was attributed to active transport. Figure 3 shows the uptake of malathion alone (control) contrasted to the uptake of malathion in the presence of inhibitors. This is in contrast to other findings (SHAH and GUTHRIE 1970). These differences may be explained by the fact that cell suspensions were utilized in this study whereas intact membranes were used in the earlier study. A cell suspension system would be expected to be a more sensitive indicator of the inhibitors than the less sensitve whole membrane system of the earlier study.

When comparisons were made between this study and a previous in vivo absorption study (AHDAYA et al. 1981), malathion and nicotine were absorbed very rapidly through the intestine in the in vivo study, twice as fast as DDT and chlorpyrifos, while in the present

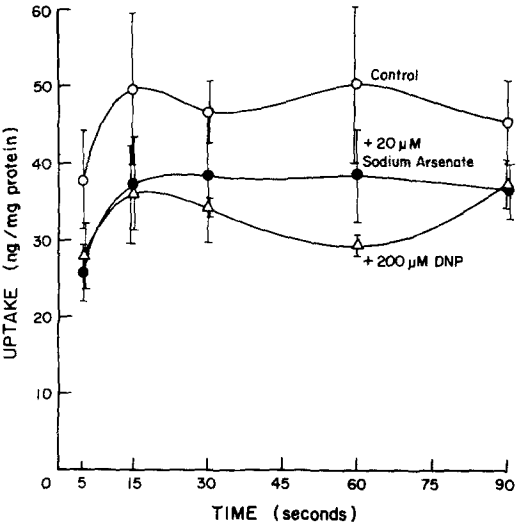



Figure 3. Effect of DNP (200 µM) and sodium arsenate (20 mM) on uptake of malathion by cell suspensions. Control ($Q_{O_2}$) was 0.57 µM $O_2$/mg protein/hr; in presence of DNP $Q_{O_2}$ was 0.14 µM $O_2$/mg protein/hr/ and sodium arsenate was 0.16 µM $O_2$/mg protein/hr.

study, cellular uptake of chlorpyrifos and DDT was at least 5 times that of malathion and 40 times that of nicotine.

Perhaps compounds with high partition coefficients, such as DDT and chlorpyrifos, are taken up rapidly as the result of the high affinity of cell membranes for such compounds but then became very tightly associated with lipid constituents in the cells, reducing their further penetration. On the other hand, compounds with lower partition coefficients are taken up less rapidly but are able to pass through the cell more easily due to their weak association with cell constituents thus facilitating rapid diffusions to other tissues. MURAKAMI and FUKAMI (1979) suggested that DDT associated primarily with lipid-rich (surface) membranes.

A better correlation between the physical properties of insecticides and their uptake in cell suspension was reported and is in contrast to in vivo absorption studies in the same animal. This leads to the conclusion

that apolar compounds penetrate most easily in simple systems (cell suspensions) but in the *in vivo* situation adequate polarity appears to be an important factor in penetration.


## ACKNOWLEDGEMENTS

Research supported by PHS Grant ES-00044. Paper No. 8202 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC.


## REFERENCES


AHDAYA, S. M., R. J. MONROE, and F. E. GUTHRIE: Pestic. Biochem. Physiol. 16, 38 (1981)

EVANS, E. M., J. M. WRIGGLESWORTH, K. BURDETT, and W. F. R. POVER: J. Cell Biology 51, 452 (1971).

KIMMICH, G. A.: Biochemistry 9, 3659 (1970).

KIMMICH, G. A.: in Methods in Membrane Biology, E. D. Korn, ed. V. 5 pp. 51-115, Plenum Press, (1975).

MARTIN, H., and C. WORTHING (eds) "Pesticide Manual", British Crop Protection Council, Worcester, England, 1974.

MURAKAMI, M. and J. FUKAMI: Bull. Environ. Contam. Toxicol. 15, 425 (1976).

MURAKAMI, M. and J. FUKAMI: Bull. Environ. Contam. Toxicol. 19, 423 (1978).

MURAKAMI, M. and J. FUKAMI: Bull. Environ. Contam. Toxicol. 21, 478 (1979).

SHAH, A. H. and F. E. GUTHRIE: Comp. Gen. Pharmac. 1, 391 (1970).

SHAH, P. V., R. J. MONROE, and F. E. GUTHRIE: Toxicol. Appl. Pharmacol. 59, 414 (1981).



Accepted April 27, 1982