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# Dermal Levels of Methyl-Parathion, Organochlorine Pesticides, and Acetylcholinesterase among Formulators

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Dermal absorption has long been recognized as a major route of exposure to organophosphate pesticides. Measurements after actual usage have been made using patch tests, hand rinses, and fluorescent visualization. Depression of erythrocyte acetylcholinesterase (AChE) has been reported in relation to oral doses of various pesticides, including ethyl- and methyl-parathion (Rider and Puletti 1969; Rider *et al.* 1970; Rider *et al.* 1971). Dermal doses have also been measured in relation to erythrocyte AChE depression in a few studies (Spear *et al.* 1977, Hayes *et al.* 1964, Hussain *et al.* 1990). One study found an average 10% depression of erythrocyte AChE (although not clinically abnormal) with an average dermal deposition during the workday of 5.5 mg methyl-parathion (Ware *et al.* 1975).

Nevertheless, information on actual dermal dose-response measures is sparse. We have investigated the potential contribution of measurable dermal methyl-parathion to erythrocyte AChE depression among formulators during a clinical screening of workers exposed to organophosphate pesticides. As a measure of dose, we have utilized a simple skin wipe technique similar to that previously employed in studies of other airborne environmental contaminants (Wolff *et al.* 1989). For field measurement of erythrocyte AChE, we used a sensitive and reproducible method that has recently become available (Magnotti *et al.* 1988; McConnell *et al.*, in press).

## MATERIALS AND METHODS

Thirteen workers, formulating methyl-parathion in a powder base, were evaluated for clinical organophosphate poisoning in Mexico during July, 1990. Erythrocyte AChE was measured in whole blood from thumb sticks using the method of Ellman as modified by Magnotti *et al.* (1988). Mean erythrocyte AChE levels in these persons, which did not differ from an unexposed comparison group, have been reported (McConnell *et al.*, in press). In order to determine the relationship between methyl-parathion and erythrocyte AChE levels, wipe samples of the palms and of a 9 $cm^2$ area of the forehead were obtained with the same alcohol wipes used to cleanse the thumb for erythrocyte AChE measurements. The wipes were stored in pre-washed (detergent, water, acetone, hexane) scintillation vials.

Because the workers reported having formulated BHC and DDT, the samples were also analyzed for these pesticides. During the analysis, chromatographic peaks corresponding to *p,p'*-DDE, *o,p'*-DDT, and *o,p'*-DDE were observed, and these were also quantified.

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Standards were obtained from the U. S. EPA Pesticides and Industrial Chemicals Repository, Las Vegas, Nevada. The vials containing the wipes were agitated and rinsed with 3 aliquots of ethyl acetate to achieve a volume for analysis of approximately 1.5 mL. This solution was analyzed directly for methyl-parathion using gas chromatography (gc) on 4%SE30-6%OV201 column (1/8 in id x 9 ft long) with a nitrogen-phosphorous detector (Perkin Elmer Sigma 1 gc). The remainder was evaporated, redissolved in hexane, chromatographed on Florisil (1.8 g, activated overnight at 130°C, collecting 18 mL 6% ether in hexane), and analyzed for organochlorines using gc (same column, electron capture detector). Recoveries of methyl-parathion were 95±9.8% (n=16), unaffected by the presence of the alcohol swab or by storage for as long as 7 days at room temperature. Other recoveries were γ-BHC 70±26% (n=6; without one outlier 79±19%); *p,p'*-DDE 83±10% (n=8); *p,p'*-DDT 101±25% (n=6; without one outlier 92±10%). Levels of interfering compounds (blanks) were negligible.

Identities of these pesticides were confirmed by gc analysis of 8 samples on a 1.5%OV17-1.95%OV210 column. From this analysis, BHC (as analyzed with the first column and reported in tables 1-2) was observed to be a mixture of the γ- and β- isomers. It was estimated from the confirmatory analyses that the quantity reported as BHC was approximately 75% of the actual γ- plus β- isomer concentrations, or 1.2 times the actual γ-, and twice the actual β-BHC levels (because of the different detector response of γ- and β- BHC). In addition, the confirmatory analyses identified α- and δ- BHC. In these 8 samples, α- was approximately three times and δ- about the same as the γ- + β- BHC isomers (from means and linear regression).

Statistics were calculated using SAS-PC provided through the City University of New York Computer Center. Spearman rank correlation ($r_s$) was used to examine pesticide interrelationships.

## RESULTS AND DISCUSSION

Eight of the thirteen workers examined reported having worked with methyl-parathion during the last 15 days; seven used BHC; and three used DDT. All bathed daily, and none laundered his work clothes more often than weekly (median every 15 days, range 3-60 days). All reported wearing masks, boots and hard hats at work. One person reported occasionally wearing gloves.

Mean erythrocyte AChE was higher among the five persons who had *not* washed prior to testing. These workers also had higher levels of all the chemicals tested than did those who had washed (Table 1). This positive correlation of erythrocyte AChE with methyl-parathion was seen in wipes of both the palm ($r_s$ 0.52 p=0.07 n=13) and the forehead samples ($r_s$ 0.65 p=0.02 n=13).

From 32 ng to 277 ug of the pesticides (methyl-parathion, *o,p'*- and *p,p'*-DDT, BHC) were measured in the alcohol wipes of the palm and forehead. The median levels of methyl-parathion for both palm and forehead corresponded to 0.01 ug/cm$^2$. Median levels of DDT and BHC were similar to those of methyl-parathion (table 1). One of the field staff who had not been working with chemicals had detectable residues of BHC only (2 ng forehead, 4.2 palm; this person is not included in the data in table 1). A laboratory worker in NYC had 0.9 ng DDE on the forehead; none was detected on the palm.

Methyl-parathion on the palm was marginally increased for those who reported having used it the same day (table 2). Otherwise the dermal contamination was not significantly related to the date of last exposure nor to laundering of clothes. Workers who did not

**Table 1:** Amounts of pesticides (ng) on the skin of formulators after the workshift and erythrocyte acetylcholinesterase (AChE) levels

| | Forehead (9 $cm^2$) | | | | | | |
|---|---|---|---|---|---|---|---|
| Washed after work? | No | | | | Yes | | |
| | n | median (ng) | range[a] | | n | median (ng) | range |
| MeP[a] | 5 | 510 | 90-11000 | *tw* | 8 | 74 | 20-200 |
| BHC | 5 | 506 | 248-1162 | *tw* | 8 | 127 | 50-558 |
| DDT | 5 | 368 | 95-877 | *t* | 8 | 92 | 22-352 |
| opDDT | 5 | 64 | 32-259 | *w* | 8 | 26 | 5.9-79 |
| DDE | 5 | 18 | 2.6-106 | | 6 | 16 | 3.3-23 |
| opDDE | 2 | | 5.1,5.9 | | 2 | | 3.1,4.4 |
| | Palm (~50 $cm^2$) | | | | | | |
| Washed after work? | No | | | | Yes | | |
| | n | median (ng) | range[a] | | n | median (ng) | range |
| MeP | 5 | 9200 | 690-277000 | *w* | 8 | 345 | 40-3000 |
| BHC | 5 | 4175 | 1571-28653 | *w* | 8 | 695 | 236-11040 |
| DDT | 5 | 1128 | 677-10470 | *tw* | 8 | 501 | 212-1129 |
| opDDT | 5 | 283 | 181-3533 | *w* | 8 | 110 | 55-170 |
| DDE | 5 | 117 | 22-1107 | | 8 | 34 | 16-98 |
| opDDE | 3 | | 20-79 | | 4 | | 6.8-86 |
| AChE[b] | 5 | 30.1 | 28.7-31.6 | *t* | 8 | 27.4 | 26.0-30.8 |

| | Ratio, Palm/Forehead[c] | | |
|---|---|---|---|
| | n | median | range |
| MeP | 13 | 5.7 | 1.1-50.3 |
| DDT | 13 | 6.5 | 1.5-32.7 |
| BHC | 13 | 4.9 | 2.5-27.4 |
| DDE | 11 | 4.4 | 1.2-45.0 |
| opDDT | 13 | 4.9 | 1.7-28.0 |

[a] MeP= methyl-parathion *t*: significantly different for washed *vs* not washed by Student's t test. *w*: by Wilcoxon rank sum which was used to compare these values

[b] International units per gram hemoglobin

[c] The ratios were not different for those who washed after work and those who did not.

wash up after the shift just prior to testing had higher pesticide levels on the skin (table 1). Among those who had not washed after work, levels of methyl-parathion on the palm were 25 times higher, and on the forehead 7 times higher, than among those who washed. The organochlorines were also higher, 1-6 times on either forehead or palm, among those who had not washed.

The forehead levels were approximately 1/5 those on the palm, regardless of washing status or particular pesticide (table 1). This ratio is approximately that expected simply on the basis of the area wiped. Levels on the forehead were correlated with those on the palm ($r_s$ 0.6-0.85 for the three pesticides), suggesting that wipes of either site may serve as a marker of exposure. The amounts of chemical on various parts of the body are relevant to exposure, since penetration differs at different sites. For example, absorption

from the forehead was approximately four times that from the forearm for parathion and other chemicals (Wester and Maibach 1989b). Hence, levels of pesticide on the forehead in these workers would add substantially to exposure from the hands.

The pesticides (methyl-parathion, *o,p'*- and *p,p'*-DDT, BHC) were significantly correlated with each other (palm: $r_s$ 0.6-0.88, forehead: 0.61-0.89, n=13). Among those who had not washed, the correlations were higher ($r_s$ 0.9-1.0, n=5). Those who had washed showed two correlations of this magnitude among the chemicals on the forehead (*o,p'*-DDT with *p,p'*-DDE or *p,p'*-DDT, n=8) but none on the palm.

The skin wipes were a rapidly and easily collected method for demonstrating exposure to methyl-parathion, the pesticide of main interest, as well as two other organochlorine pesticides. A wide range of amounts deposited was observed. The highest levels of methyl-parathion as well as the other chemicals in Table 1 (1-3 ug/cm$^2$) are consistent with other dermal exposures that have been reported. Those estimated by Wester and Maibach (1985) for topically applied thin films ranged from 4 ug/cm$^2$ for a dilute solution to 1000-2000 ug/cm$^2$ for neat pesticide. Persons working in previously sprayed cotton had 5.5 mg (average) methyl-parathion on the exposed skin plus 50.5 mg on the shirt and pants (Ware *et al.* 1975). Under similar work circumstances, other investigators reported 0.7 mg/hr dermal deposition (Wolfe *et al.* 1967). Kazen *et al.*(1974) reported 0.1-39 ug of ethyl-parathion, obtained by rinsing the hands of farm users with solvent. A number of other studies during actual use of organophosphate and carbamates have reported dermal deposition rates of <1 ug per day to 78 mg/hr using patch and hand-rinse techniques. Since the levels in our study were determined after workers had left the workplace, it may be assumed that during actual pesticide formulation skin levels were higher than those measured. This is one of the disadvantages of this method compared with dermal patches that measure accumulated dermal deposition of airborne pesticide. This disadvantage can sometimes be overcome by obtaining skin wipes before work begins (measuring retention from the previous day) or by insuring that all subjects

**Table 2:** Skin levels of pesticides with respect to reported most recent exposures

| most recent exposure | n | forehead median | forehead range | palm median | palm range |
|---|---|---|---|---|---|
| | | Methyl-parathion (ng) | | | |
| same day | 4 | 1000 | 90-11000 | 6100 | 920-277000[a] |
| 1-15 days | 4 | 74 | 47-510 | 525 | 259-1180[b] |
| >15 days | 5 | 90 | 20-300 | 330 | 40-15100[c] |
| | | BHC (ng) | | | |
| same day | 3 | 558 | 137-947 | 4175 | 535-11040 |
| 1-15 | 4 | 97 | 72-248 | 933 | 565-1571 |
| >15 days | 6 | 317 | 50-1162 | 1369 | 236-28653 |
| | | DDT (ng) | | | |
| <15 | 3 | 95 | 76-415 | 677.0 | 328-1084 |
| >15 days | 9 | 102 | 22-877 | 567 | 212-10470 |
| | | *o,p'*-DDT (ng) | | | |
| <15 | 3 | 32 | 30-89 | 181.0 | 143-250 |
| >15 days | 9 | 32 | 5.9-259 | 143 | 55-3533 |

a *vs* b + c (methyl-parathion the same day versus any time earlier): Wilcoxon p=0.10 (forehead) p=0.054 (palm). Exposure times for BHC and DDT did not differ significantly.

observe similar personal hygiene (washing-up) after work.

The most significant predictor of skin levels was personal hygiene: washing up after work. Washing reduced the levels of all of the observed pesticides, but the reduction was more marked for methyl-parathion than for the organochlorines. This may have been simply because the methyl-parathion was present in relatively larger amounts, due to recent use. Indeed, the ratio of methyl-parathion to the other pesticides was much larger for those who had not washed, on either forehead or palm. Methyl-parathion may also be more readily removed because it is less bound to skin, or less lipid soluble, than the organochlorines. And similarly, its water solubility may allow it to be more easily removed by washing than the others. Cleansing can remove 10-90% of topically applied chemicals from the skin, depending on how soon washing occurs and on the individual substance (Wester and Maibach 1985; Wester and Maibach 1989a). However, even though the skin wipe levels are lower, dermal absorption may still be high. Thus, washing at varying intervals in experimental studies did not markedly reduce absorption (Wester and Maibach 1985), so that the actual body burdens of pesticide formulators may be quite similar for those who have and have not washed.

DDT and BHC were also formulated at this site. The similarity of dermal levels of these and methyl-parathion suggests that widespread contamination existed with all of them. Persistence and carryover of the chemicals on the skin is also suggested by the uniform presence of the chemicals on the skin of these workers. The levels of *o,p'*-DDT, approximately 25% of *p,p'*-DDT, were consistent with the expected level of contamination of technical DDT.

The source of DDE is of particular interest, since this residue has been attributed almost exclusively to metabolic conversion of DDT (ATSDR 1989). Therefore, it could arise from endogenous DDE. Endogenous DDE excretion in forehead skin lipids in an earlier study was 0.2-18 ng (median 1.3 ng) from a 9 $cm^2$ area for normally exposed persons (Wolff 1984). Persons occupationally exposed to DDT have 2-10 times higher body burdens than normal (Morgan *et al.* 1980). Therefore, the range of DDE levels in Table 1 are consistent with occupational exposure, being approximately 10 times normal values (median 18 ng over 9 $cm^2$ forehead; 38 ng over 80 $cm^2$ palm compared with "normal", 1.3 ng over 9 $cm^2$ [Wolff 1984]). If the source of DDE were endogenous, it would also be consistent with the observation that DDE was lower than the other pesticides tested, showed a much smaller differential due to washing than the other pesticides, and was not strongly correlated with other pesticides that presumably had been recently deposited. However, it might be predicted that levels arising from endogenous stores would be higher on the lipid-rich forehead, which is not the case (table 1). The relative levels of DDE were higher on the palm than on the forehead, just as were the other chemicals. It is theoretically possible that DDT dehydrochlorinate to DDE on the hands through cutaneous metabolic, photolytic or microbial processes. The latter has been reported for soil (ATSDR 1989). Although such processes seem unlikely to account for the levels of DDE seen here on human skin, they cannot be ruled out.

The positive association between dermal methyl-parathion residues and erythrocyte AChE levels was the opposite of that expected. (Erythrocyte AChE should decrease as dermal exposure increases.) The positive correlation of erythrocyte AChE with dermal methyl-parathion levels could be spurious, although the distributions are also strikingly different among those who did than those who did not wash after work (figure 1). It is likely that those most heavily exposed, knowing with what they had been working, tended to be more careful about washing afterward. The cumulative exposures or those earlier in the day among those more heavily exposed could have caused the observed minor depression in erythrocyte AChE while washing lowered the post-shift skin

contamination. Alternatively, it has been suggested that production of erythrocyte and plasma cholinesterase is stimulated during exposure to organophosphates (Hayes and Laws, 1991). It is possible that those with higher methyl-parathion skin levels would have subtly higher erythrocyte AChE activity as a result of increased cholinesterase production.

The dose absorbed by these workers may not have been sufficient to effect a lowering in erythrocyte AChE. On the entire body, the total deposition on exposed skin can be estimated to have been approximately 11-33 mg of methyl-parathion, using the maximum levels we observed (1 - 3 ug/cm$^2$) and 11,000 cm$^2$ for the area of exposed skin on the arms, trunk and head (Guy and Maibach 1989). (This approximation uses the highest values we found on an unwashed worker; the surface area may be exaggerated, since all these persons wore shirts.) From these figures, the maximum absorbed amount is calculated to be 3 mg or less (using the reported extent of absorption for ethyl-parathion of 1-9%; Wester and Maibach 1985; WHO 1986). Since our estimates are made from residual dermal contamination after work, the actual dermal absorption may be higher than 3 mg. This is well below the estimated level of effect for methyl-parathion, 24 mg/day orally, which resulted in a maximum mean decrease in AChE from baseline of 22% (Rider *et al.* 1971). However, it is similar to the dose of 1-6 mg absorbed estimated from another study of actual dermal exposure where maximum mean erythrocyte AChE depression was 10% (Ware *et al.* 1975).

Although all the subjects we tested had erythrocyte AChE levels within the inter-individual normal range (24.9-31.7 IU/g Hgb), inter-individual variability in erythrocyte AChE is known to be large (Hayes and Laws, 1991). However (as described in McConnell *et al.*, in press), the mean erythrocyte AChE among these workers (28.5 IU/gm Hgb; S.D. 1.98; n=13) was very similar to that of a group of plant personnel not exposed to pesticides (28.0 IU/gm Hgb; S.D. 2.37; n=10). With this inter-individual variability (< 9%), a mean inter-individual depression of 10% in AChE certainly would have been detectable in our study. However, baseline enzyme activity was not available for evaluating percentage decline, so that comparison with results from Ware *et al.* (1975) and Rider *et al.* (1971) using intra-individual change in erythrocyte AChE was not possible.

Further possible reasons for the absence of abnormal erythrocyte AChE include low exposure to methyl-paraoxon (a degradation product of methyl-parathion that is a more potent erythrocyte AChE inhibitor). Methyl-paraoxon levels may be insignificant in formulation work compared with levels in a recently sprayed outdoor area, where methyl-paraoxon can amount to 2-15% of methyl-parathion (Ware *et al.* 1975).

Another factor leading to more moderate exposures in our study could be that the pesticide formulation used in this workplace was 3% dust. Extrapolating from previous work (Wester and Maibach 1985), a 3% liquid solution of pesticide should correspond to dermal surface contamination of 50 ug/cm$^2$, which would approximate an absorbed dose of 50 mg, well above the threshhold (24 mg) for lowering erythrocyte AChE. However, formulation as a dust may cause much lower effective skin contamination than liquid solution. Dermal exposure for malathion and ethyl-parathion has been reported to be lower during application of dust than by aerosol or spray (Wolfe *et al.* 1967). An accidental exposure during our field survey further supports the idea that the maximum dermal exposure to dust is much lower than that to liquid. We observed 13 ug/cm$^2$ on the thumb of a man who had just removed his hand from the dust preparation (87 and 74 ug on two successive wipes of approximately 6 cm$^2$). Of course, the dust formulation may permit more effective respiratory absorption of pesticide, but these workers reported

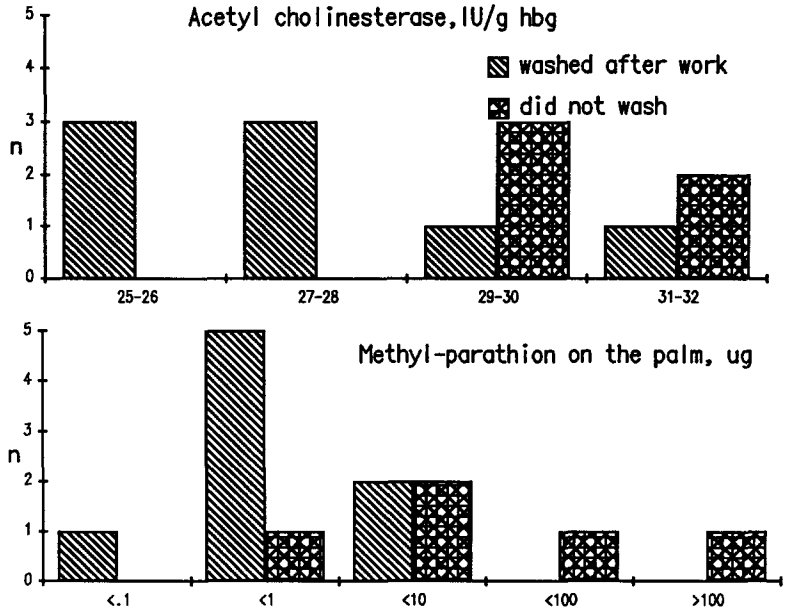


Figure 1: Acetylcholinesterase and methyl-parathion on the palm of workers who washed or not after work, according to most recently reported use of methyl-parathion.

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wearing masks during their work. Moreover, dermal exposure during ethyl-parathion powder application was still far greater than respiratory exposure (Spear *et al.* 1977).

It is also possible that exposure of these persons to organochlorines (DDT and BHC) had enhanced their ability to metabolize methyl-parathion and that a lower biologically available dose resulted (Hayes and Laws, 1991).

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