Windham, G.C. (1996). The genetic toxicity of malathion: Results of an epidemiologic study in agricultural workers and in vitro laboratory studies, California Department of Health Services.

EXECUTIVE SUMMARY

Malathion is an organophosphate chemical widely used because of its broad spectrum pesticide activities and relatively low toxicity to humans. The aerial application of malathion over large urban populations in Southern California as a part of a Mediterranean Fruit Fly Eradication Program raised concerns about potential adverse health effects. Reports in the literature of cytogenetic damage and point mutations being caused by malathion raised questions about its potential to cause genetic damage in humans. Therefore, investigators from the California Department of Health Services and the University of California, Berkeley studied micronucleus formation in human lymphocytes as a biomarker of genotoxicity *in vivo* and *in vitro*. In addition, mutation frequencies were assessed using the glycophorin-A (GPA) assay.

State workers who worked in the medfly eradication programs in Northern and Southern California which involved application of malathion as a ground treatment, were studied in 1992 and at the start and end of the season in 1993. The 1992 pilot project of 17 workers (13 "exposed" and 4 controls) showed that 50% of the applicators had detectable malathion metabolites in post-shift urine samples. The mean micronuclei level was increased in lymphocytes of exposed workers compared to controls (20.1 ± 7.1 vs. 1.43 ± 7.2 respectively, p = 0.09), but based on small numbers. Therefore, additional workers were studied the following year in September (n=34) and December (n=20). Neither of these cohorts showed a higher level of micronuclei in workers reporting exposure to malathion in the past six months compared to unexposed (combined estimates 18.5 ± 8.6 vs. 20.6 ± 9.1, respectively). Also all three cohorts were pooled, excluding duplicates, to increase sample size. Overall there was no difference in micronuclei levels by malathion exposure. Adjustment by multiple regression modeling for age, sex, smoking and alcohol consumption did not affect the results. Those workers with indicators of higher exposures (by hours of use or urinary metabolite level) did not have higher mean micronuclei levels. The GPA variant frequency was not associated with malathion exposure in any of the cohorts.

Workers also used the pesticide, diazinon, so we attempted to examine its relationship to the cytogenetic measures, and to control for any effect. In the 1992 cohort, diazinon exposure was even more strongly associated with an increased micronucleus frequency than malathion exposure, but again this was not confirmed in 1993 cohorts. There appeared to be a slight association of GPA variant frequency with reported diazinon exposure. But this was driven by a single high value in the 1993 cohort.

For *in vitro* studies, human lymphocytes were cultured either as whole blood or after Ficoll isolation and treated with malathion in doses from 5 to 100 _g/ml for 48 hours. A significant increase in the frequency of micronucleated cells (47.5/1000 versus 16.0/1000 in DMSO control, $p<0.001$) was found in isolated lymphocytes at the highest dose level (100 _g/ml) as well as at 75 _g/ml ($p<0.01$), concurrent with cytotoxicity and a strong inhibition of cell proliferation (assessed as replicative index, $p<0.001$). Many of the high dose treated cells also possessed

multiple micronuclei. Antikinetochore-antibody staining revealed that the majority of malathion-induced micronuclei were kinetochore-negative, suggesting chromosome breakage. Results from whole blood cultures showed a trend towards an increase in micronuclei level with increasing malathion dose, but it was not consistent by donor and the elevation was lower than in isolated cells.

There were several limitations of these studies, including small sample size. Based on our results it appears that malathion has a relatively low potential to cause chromosome damage *in vitro* (as measured by the micronucleus assay), and corresponding doses are much higher than ones that even professional applicators would be likely to be exposed to at work. Therefore, the potential risk of chromosome damage from malathion exposure in vivo would be relatively low. However, we do not have data on susceptible sub-groups. Our results also do not suggest a metagenic effect of malathion in vivo based on the glycophorin-A assay. More studies are needed to assess the possibility of interaction of malathion with other pesticides through combined exposure. In addition, the sensitivity of the micronucleus assay in human lymphocytes requires further study. Other measures of genotoxicity (e.g., chromosome aberrations or breaks) may be more appropriate, but are logistically more difficult to apply in population-based studies.