MONSANTO BIOHAZARDS COMMITTEE

Minutes of a Meeting

August 24, 1983

Committee members present were: Dr. George Roush, Monsanto; Dr. Leon Golberg, Duke University; Dr. Marvin Kuschner, School of Medicine, State University of New York at Stony Brook; and Dr. Robert E. Olson, University of Pittsburgh School of Medicine.

The meeting was called to order at 9:00 a.m. by Dr. Roush. Corrections to the minutes of the July 27 meeting were as follows: on page 2, paragraph 2, line 21 the sentence should read "These CD-1 mice also were affected by amyloidosis in the controls (76%) as well as the experimental groups (90%);" page 2, paragraph 3, line 3 should read ".... alarming to the Committee because it suggested that the compound is a primary carcinogen in that it produces unexpected tumors such as those of the stomach and nasal turbinates;" page 3, paragraph 1, line 6 the sentence should read "the overall view of the Committee was that the findings raised serious questions about the toxicity of Alachlor. The case for continuing use of Alachlor is based on the facts that ...;" page 3, last sentence, paragraph 1 "The Committee requested a reexamination of all the data on worker exposure.;" page 3, paragraph 3, the last sentence should read "... as a step in obtaining a more accurate view of the action of the compound." With these amendments the minutes were approved.

The first item of business was a continuing discussion led by Dr. T. Fuhremann on the toxicology of Alachlor a substituted chloroacetanilide (Lasso®). Again in attendance were Mr. Robert Harness, Director of Environmental Operations for the Agricultural Products Company and Dr. Edward Debus, Director of Product Registration for the Agricultural Products Company. Mr. Harness reported that the top executives of the Agricultural Products Company, Mr. Donald Swan and Mr. Nick Reding, had given top priority to the registration of Lasso® in the U.S. for expanded use which requires the approval of the EPA and reregistration of the product in Canada. A hearing will take place on October 6 before representatives of the Health Protection Branch and Agriculture, Canada in Ottawa under the Chairmanship of Ian Munro.

Prior to this meeting, Dr. Fuhremann had circulated to the Committee a complete file on the properties of Alachlor, including its chemistry, toxicology, metabolism and data on exposure to workers. At this meeting he proposed to review the data on the investigation of worker exposure. He reported that 70 employees of the Company had been examined for evidence of eye disease when the findings of uveal degeneration in pigmented rats was discovered some years ago. No eye disease nor other signs of ill health was found. Several of the reports in the Company file,

alluded to above, regarding exposure of workers were discussed. The study of aerial and ground applicator exposure under field conditions by Lauer and Arras was reviewed. This study found that the total body dose computed from inhalation and dermal absorption during tank fill and application, was 13.64 µg/kg body weight/ hundred acre application. On this basis about 1.0 mg would be taken up by such workers in the course of spraying 100 acres, which is about the amount that one worker could do in a day. The highest total body dosage per pound of Alachlor applied was 0.034 µg/kg body weight. This worked out to be 0.02 µg/kg body weight/day over a lifetime, which in turn computes to a lifetime risk of developing a tumor based on the animal studies of about 10^{-8}. This is done in accord with the EPA mode of calculation in which the acceptable risk is usually put at a chance of 10^{-6} of developing a tumor in a lifetime.

It is known that chloroacetanilides in general are skin sensitizers that result in skin rashes with/without burning and itching. In studies of skin penetration of Alachlor in monkeys dosed with 12 mg per 8 cm^2 of skin, 15% of the Alachlor applied was excreted in the urine over a 5 day period with a t 1/2 of 31 hours. Approximately 70% of parenteral doses appear in the urine, with smaller amounts in the stool, over a 5 day period. Urinary measurements appear to be a good way of determining uptake of the compound by man from the environment.

When human cadaver skin is studied in a diffusion chamber it is found that only 1% of acetanilide crosses the barrier, but this of course is not a circulated organ, and may not represent the quantitative potential of normal human skin to take up the compound. It was concluded from many studies that dermal uptake is probably the main route for uptake of Alachlor by man. No data, however, are available on plasma and urinary levels of the compound in human beings exposed to this material. It was recommended by the Committee that such studies be undertaken with sampling of urine at various times after exposure of workers to the compound under field conditions.

The risk assessments which have been made thus far on the compound are dependent upon assumptions about air content and dermal uptake. The study of the urinary excretion of Alachlor metabolites would provide a better estimate of the dose taken up, which in turn would aid in refining risk assessments.

The next topic discussed was the metabolism of isotopically labelled Alachlor in rats. A large variety of metabolites have been identified which are derivatives that retain the basic acetanilide structure with conjugation to thiols. Fourteen compounds identified in the urine all contain substituted thiols presumably originally derived from glutathione. Some are further oxidized to sulfones, sulfoxides and sulfates. Two were glucuronic acid conjugates. Some of the compounds were degraded further to 4-amino-3,5-diethylphenyl sulfate and compounds

hydroxylated in the two position of the ethyl side chain. In some compounds the nitrogenous side chain bearing the methoxymethyl group is eliminated but in general the acetanilide structure is preserved. This biochemistry is discussed more completely in the information circulated to the Committee.

Dr. Golberg asked how the doses for testing were selected, above 42 mg/kg (equivalent to approximately 1000 ppm). The dosage levels were initially set on a mg/kg basis (14, 42, 126) to be equivalent to the dietary concentration (100, 300, 1000 ppm) used in previous Industrial Biotest studies. However, as the animal gained weight and ate proportionately less treated diet, the dietary concentrations were increased nearly 3-fold to maintain the initial dosages. Administering compounds in the diet in terms of ppm of diet is equivalent to administering compounds in mmoles/calorie which correlates better with surface area and energy expenditure rather than with body weight. Adjusting the dose for body weight, taking into account changes in food intake and body weight, will give a higher dose than maintaining a constant percentage in the diet. It is clear now that 126 mg/kg is beyond the maximum tolerated dose because of weight loss, increased mortality rates, and enlargement of liver and other organs.

Dr. Golberg argued that a very important set of experiments should be done to study the distribution and metabolism of Alachlor at both high and low doses. It was his belief, agreed to by the Committee, that the pattern of metabolism might be quite different at high doses as compared with low doses and it would be important to determine the patterns under the two conditions of animal feeding. It would also be important to study the uptake of the compound and metabolites by various organs that have been observed to have pathologic changes under high dose feeding, such as the thyroid, the stomach, the nasal turbinates, the lung and the brain. Since 90% of Alachlor and its metabolites are excreted in the urine and feces in 4 days it would be important to determine the dose response curves for Alachlor administered in various ways to animals in preparation for doing similar studies in man. It was recommended that hepatic glutathione levels be measured under high and low dose feedings because of the high percentage of sulfur conjugates which have been identified so far.

Dr. Golberg also recommended that the degree of hemoglobin and DNA derivatization by Alachlor or its metabolites be measured as an index of covalent modification of macromolecules by the compound, which could bear on its action as a carcinogen. A paper by Werner K. Lutz (Banbery Series, Session IV: DNA Adducts, 189-204) from the Institute of Toxicology, Zurich, entitled Covalent Binding Index - DNA Binding in Vivo as a Quantitative Indicator for Genotoxicity, which summarizes recent advances in this area measuring carcinogenic potency by determining DNA binding was cited by Dr. Golberg.

It was agreed that the Company scientists concerned with the study of Alachlor metabolism and related issues would circulate to the Biohazards Committee within the next two weeks all the data available on metabolism and exposure of workers and would prepare a draft of a program to systematically study the toxicology of Alachlor for discussion at the next meeting of the Biohazards Committee which will occur on September 15-16, 1983 beginning at 2:00 p.m. following the Monsanto Symposium on New Approaches in Toxicity Testing and Their Application to Human Risk Assessment.

Item two on the agenda "Uncoupling Chemicals and Cancer" was suggested by Dr. Richard Mahoney, the new President of Monsanto and Mr. Joe Nolan, Vice President for Public Relations. Could the Company carry on a campaign to educate the public about the true role of chemicals in biology and to disabuse laymen of the view that all chemicals are toxic. Since living tissues are composed of many chemicals, and the maintenance of life is dependent upon chemical reactions that generate energy, which in turn is required for the performance of work by all living forms, chemicals are essential for life. Only selected chemicals can injure or alter the expression of genes which in turn can lead to a change in the phenotype of cells, so-called transformation of cells. It was agreed by the Committee that this is a very worthwhile project and that the Company should invest some money in carrying out a campaign of this kind. The question is how to do it. Should this effort center about the appointment of professors in large or small institutions of higher learning, who would devote more of their time to teaching the biochemistry of cancer, and the role of environment. Some one should put into perspective the various risks that face human beings on this planet, including the risk of cancer. Dr. Olson suggested that the American Council on Science and Health might be a useful vehicle for getting informative papers into popular and semi-popular journals. Dr. Beth Whelan is Executive Director of this organization.

The next item on the agenda was a discussion of a newly discovered epidemic of purported hemoglobinuria in workers at the Pensacola Plant. No red cells, however, were found in the sediment. The positive tests for hemoglobin were based on the benzidine test. The average prevalence of positive hemaglobin tests by this method in the urine of Company employees is about 3%, whereas in Pensacola 400 out of 4400 people or about 8% were positive. This positive test, however, was transient and in urine standing 6 to 8 hours the chromagen disappeared. Urine spiked with hemoglobin will remain positive for many hours. Although it seems that these results are false positives, the question is what or who is responsible? By preliminary gel filtration of the 200 mg of protein found normally in a 24 hour urine, the offending material was adsorbed to a protein of about 60,000 molecular weight, which could be albumin. This fraction should be tested for the spectrum of hemoglobin or hematin. If negative, the reacting substance should be isolated. Distribution of positive urines is not segregated to one part of the factory

or any one process. It is distributed among workers and executives but is not present in the general population. Could it be a component of the diet served at Pensacola? The problem is under investigation.

The next item was the issue of dioxin-related soft tissue sarcomas presented by Dr. William R. Gaffey. Of the soft tissue sarcomas that have been associated with deaths of workers at Monsanto, are they related biologically? Of the five cases which we examined it was clear that the tumor in one case, that of David L. Cluck who had worked for the Company for only two years and who died at age 34 of a metastatic mesothelioma, could not have arisen because of occupational exposure. Of the four remaining cases two had liposarcomas and one a fibrous histiocytoma which could be considered related. The malignant schwannoma which is tissue of a different origin and different cell-type is clearly different. As the cell-type in these sarcomas varies considerably, it was Dr. Kuschner's opinion that three of the four might be related. The Committee requested that slides be obtained from these patients for examination by Dr. Kuschner and the Company agreed.

The next item was a discussion of advances in the understanding of malignant transformation particularly as reflected by recent papers in the New England Journal of Medicine (T. G. Konteres: Emerging Genetics of Human Cancer, NEJM 309, (August) 404, 1983) and the Lancet (P. Hamlyn and K. Sikora: Oncogenes, Lancet II, 326, 1983). Should knowledge of oncogenes affect the attitude of the Company toward carcinogenesis? The new concepts illuminate the process by which expression of new genes occurs to change the phenotype of normal tissue to tumor. It should lend support to continuing studies of DNA modification by compounds of interest to the Company, such as suggested today for Alachlor.

The sixth item was a report by an ad hoc working group on oil gavage in toxicology sporsored by the Nutrition Foundation. It was a review of current literature by an expert Committee chaired by Dr. Jelliffe Carr of Columbia, Maryland and composed of a number of toxicologists known to the Committee. They concluded that corn oil gavage may not be the best vehicle for administering carcinogens to rats because of several features. Corn oil: 1) may change the distribution of the compound between oil and other fluids of the gastrointestinal tract which may promote absorption of compounds normally degraded by digestive enzymes; 2) may increase peak loads in the blood; 3) adds calories to the intake of the experimental animal; and 4) is not to be recommended for NCI type long-term carcinogenesis studies.

The seventh item on the agenda was a consideration of a request from the Rene Dubos Foundation in New York for funds to support symposia which permit interaction of industry scientists, and consumer advocates. The Committee viewed this project with some skepticism since there has been no program set forward and no topics defined. Because of the vagueness of the proposal and

the lack of definition the Committee recommended that the Company not support this proposal.

Jim Wilson touched on two points. He wanted the Committee's opinion on a press workshop to try to improve the understanding of the working press about the Company's attitude toward carcinogenesis and their attempts to institute good occupational health practices and a good toxicology program. The Committee supported that. The other proposal was that Dr. Alan Poland, a biochemist at the McCardle Institute for Cancer Research, University of Wisconsin-Madison, be invited to come to Monsanto and talk about his studies on receptors for dioxins. The Committee, all of whom know Alan Poland, agreed that he is an excellent speaker and recommended that he be invited to present a seminar at Monsanto at some future meeting of the Biohazards Committee.

The meeting was adjourned at 3:00 p.m. The next meeting will occur on September 15-16, 1983.

Robert E. Olson, M.D.
Secretary, Monsanto
Biohazards Committee