FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL MEETING

A Set of Scientific Issues Being Considered by the Agency in Connection with Chlorothalonil: Mechanism for the Formation of Renal and Forestomach Tumors.

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) has completed its review of the set of scientific issues being considered by the Agency in connection with Chlorothalonil: Mechanism for the Formation of Renal and Forestomach Tumors. The review was conducted in an open meeting held in Arlington, Virginia, on July 30, 1998. The meeting was chaired by Dr. Ernest E. McConnell (ToxPath, Inc.) Other Panel Members present were: Dr. Rory Conolly (Chemical Industry Institute of Toxicology-CIIT); Dr. Michael Cunningham (National Institute of Environmental Health Sciences-NIEHS); Dr. Amira Eldefrawi (University of Maryland School of Medicine); Dr. Gordon Hard (American Health Foundation); Dr. Genevieve M. Matanoski (The Johns Hopkins University); Dr. Fumio Matsumura (University of California) and; Dr. Christopher Portier (National Institute of Environmental Health Sciences-NIEHS.

Public Notice of the meeting was published in the Federal Register on June 19, 1998.

Oral statements were received from the following:

Dr. William Busey (Environmental Pathology Laboratories, Inc.)

Dr. John Foster (Zeneca)

Dr. Ashley Wickramaratne (Zeneca)

No written statements were received.

General Comments from SAP Members

Several points were raised during the Agency presentation, public comment, Panel general discussion, and response to Agency questions that represented issues or viewpoints on the chlorothalonil deliberation. In particular, several aspects related to chlorothalonil's activity in the rodent kidney were covered during general Panel discussion.

1. In response to questioning by the Panel, the Agency stated that tubule cytotoxicity in the rat kidney commenced at a very early time-point and was sustained through the period of compound administration, matching data available on increased tubule cell proliferation. Cell proliferation studies had not been conducted in the dog, but chlorothalonil did not induce histopathological changes in this species.

2. The Panel noted that a CD-1 mouse study, positive for renal tumors, had not been included in the Agency's data presentation.

3. Discussion on tubule hyperplasia, recognized as a precursor lesion to renal tubule tumors, confirmed that it preceded tumor development and had been observed in interim and subchronic studies in a dose-response pattern. Based on experience with other renal carcinogens (genotoxic and non-genotoxic), the incidence of chemically-induced renal tubule hyperplasia would not be expected to be 100 percent in any given study.

4. The Panel considered information on the activity of γ-glutamyltranspeptidase (GGT) in the kidneys of neonatal rats and healthy human fetal tissue compared with adult activity for each species. Whereas neonatal rats possessed activity twice as that of mature rats, human fetal tissue contained less GGT activity than adults, suggesting that infants may be less susceptible to chemicals acting through this metabolic pathway. However, this was a single study and no comparative species information exists for β-lyase at immature ages. More plentiful data have been recorded on GGT activity in the plasma of infants, but the derivation of plasma GGT activity is from liver and not kidney. β-lyase was likely to be an inducible enzyme but GGT was probably not inducible in the kidney because it is localized to the brush border of renal tubules.

5. Attention was drawn to an *in vitro* study using the β-lyase inhibitor, aminooxyacetic acid, which exerted no modifying effect on chlorothalonil toxicity. This result might suggest that the β-lyase pathway was not involved in chlorothalonil metabolism. Additional information, however, indicated that the concentration of chlorothalonil used in this *in vitro* study represented an overwhelming and lethal dose. On the other hand, research from several laboratories with halogenated alkenes/alkanes has indicated that involvement of the cysteine conjugate β-lyase pathway can lead to formation of thiols that are electrophilic agents capable of reacting with DNA.

6. The Panel expressed the position that data analysis consider the dose-response curves for renal tumors and tubule hyperplasia to determine whether the multiple dose-points fitted a straight line or not. Likewise, it was considered important to evaluate the dose-response behavior for cell proliferation. Furthermore, the draft EPA cancer risk assessment guidelines suggested use of a benchmark dose analysis to select the point-of-departure. The suggested point-of-departure in the rat could then be transformed into a human equivalent dose.

7. Another point concerned a positive *in vitro* comet assay using human peripheral blood lymphocytes in which chlorothalonil produced a positive result indicative of single-and double-strand DNA breaks and alkaline-labile sites. Several Panel members considered that the result of this study should be discounted because of the toxic doses employed and the lack of accompanying data on *in vivo* exposure levels.

8. A number of concerns related to chlorothalonil activity in the kidney were raised by a Panel member. One aspect concerned published *in vitro* data on rat hepatocytes indicating that chlorothalonil may have the capability of producing cytotoxicity by a mechanism involving oxidative stress. The Panel member acknowledged that there was sufficient precedence in the literature to support the conjugation of chlorothalonil with glutathione (GSH) in the intestine, but that data were not available to demonstrate whether this was the only metabolic pathway for chlorothalonil. It has been reported that 30% of the radioactivity in rat urine following

administration of ^{14}C-chlorothalonil represents di- and tri- thiol metabolites and their methylated derivatives (produced by further metabolism of the cysteine conjugates through the β-lyase pathway), but the remaining 70% of radioactivity was yet to be identified and characterized. The Panel member considered there to be no available evidence inferring that the GSH conjugates of chlorothalonil were nephrotoxic. The member also registered concern that, at least with another chemical, 2-bromo-(diglutathion-S-yl)-hydroquinone, the differences between species in renal activity of GGT did not correlate with the species susceptibility to the toxicity of this compound. For example, guinea pigs possess even less GGT than do humans, but were very susceptible to the renal toxicity of 2-bromo-(diglutathion-S-yl)-hydroquinone, indicating the activity of GGT may not be related to the toxicity of the metabolite. The Panel member concluded this may have implications for chlorothalonil.

9. In summary, there was a majority view of Panel members that the cytotoxicity/regenerative cell proliferation pathway was plausible and the likely mode of action for chlorothalonil. In addition, risk assessment based on differential enzyme activities in rats vs. humans is not appropriate. It was agreed that data gaps exist for chlorothalonil on such points as identification of the toxic metabolite(s), their potential for accumulation in the kidney, and potential for binding to DNA. However, one Panel member concluded that there was no compelling evidence that chlorothalonil carcinogenicity is mediated through a cytotoxic mechanism, nor was there evidence that it is cytotoxic via the proposed GSH metabolite. The one Panel member differed with the remainder of the Panel and concluded that risk assessment based on the differential enzyme activities in rats vs. humans is appropriate.

Questions to the Scientific Advisory Panel

The Agency posed the following questions to the SAP regarding Chlorothalonil: Mechanism for the Formation of Renal and Forestomach Tumors.

1. Based on our review of the dose response data, does the Panel agree that the proposed mode of action for chlorothalonil is scientifically reasonable, valid, and supported by the data?

There was a majority view amongst Panel members that the mode of action for chlorothalonil's activity in the rodent kidney, based on sustained cytotoxicity and regenerative cell proliferation during compound administration, as presented by the Agency, was plausible and likely to be valid. In this respect, chlorothalonil appeared to be acting in a mode similar to chloroform in the rodent kidney. However, it was acknowledged that many data gaps still exist, particularly related to the identity of the ultimate metabolic end-points and their ability or not to react with renal tubule DNA. There was no consensus on whether the mechanism of cell death induced by chlorothalonil occurred only through disruption of mitochondrial respiration. One dissenting view held that data from *in vitro* and *in vivo* studies could be interpreted as indicating chlorothalonil to be a DNA-reactive agent causing DNA damage and cell death through an oxidative mechanism. Such a view would increase concern about the potential carcinogenicity of chlorothalonil for humans and warrant risk assessment by use of a linearized low-dose extrapolation model.

For the rodent forestomach tumors induced by chlorothalonil, there was general agreement amongst the Panel that chlorothalonil was similar to a group of non-genotoxic rodent forestomach carcinogens that were either mucosal irritants or disruptors of the gastric mucosal barrier, causing repeated injury to the forestomach lining with inflammation, and sustained increased cell proliferation. This leads to hyperplasia and ultimately, neoplasia, representing an indirect or secondary mechanism of carcinogenesis.

2. Based on the proposed mode of action, is a non -linear approach for risk assessment appropriate. The proposed mode of action points towards an MOE approach. Does the Panel agree?

Assuming a mode of action involving sustained cytotoxicity and regenerative cell proliferation, a margin-of-exposure (MOE) approach would be in order. However, a view was expressed that the rodent data should be analyzed with, for example, the Weibull model to determine whether the data points adhered to a linear or non-linear pattern. This view held that the exercise would guide a sounder scientific and public health decision.

3. If the Panel agrees to the MOE approach, is the selection of the 1.5 mg/kg/day dose level an appropriate point of departure?

Several studies have pointed to 1.5 mg/kg/d being an appropriate point-of-departure based on the lack of forestomach lesions and an absence in the kidney of increased tubule cell proliferation and hyperplasia. However, the Panel believed that this question could only be answered after non-linearity had been tested by an appropriate statistical model, as recommended in the response to Question 2. One Panel member strongly recommended the use of a benchmark dose rather than a no-observable-adverse-effect-level (NOAEL) for determining the point-of-departure dose.

4. One of the important aspects of the proposed mechanism of chlorothalonil-induced renal tumors is the involvement of one or more enzymes involved in the metabolism of chlorothalonil to nephrotoxic metabolites. Data presented suggest species differences in activities of gamma-glutamyl transpeptidase (GGT) and cysteine conjugate β-lyase between rats and humans such that humans may be less sensitive to nephrotoxicity of chlorothalonil. Does the Panel agree?

There was a general view from the Panel that the research indicating quantitative differences in γ-glutamyltranspeptidase (GGT) and β-lyase activity between rats and humans underlined the plausibility that humans may be less susceptible than rats to renal carcinogens acting through metabolic pathways involving these enzymes. One Panel member was less convinced because the data supporting this mechanism are incomplete and mice have less GGT activity than rats and only about twice the amount present in human kidney. In addition, this species still showed a carcinogenic response, albeit weak.

5. The Agency is not in possession of any data to suggest whether the activities of renal GGT and cysteine conjugate β-lyase are significantly different in human infants and children from that of adult humans or animals. Does the Panel have any comment on the relative activities of GGT and cysteine conjugate β-lyase among animals and humans, and whether potential differences in the response of the kidney to nephrotoxicity of chlorothalonil should be expected among human subpopulations?

Although data showing lower levels of GGT in human fetal kidney tissue might suggest a lower susceptibility in the young compared to adults, it was generally agreed that not enough information existed on this aspect to provide a meaningful answer to the question.

FOR THE CHAIRPERSON:

Certified as an accurate report of findings:

Paul I. Lewis

Designated Federal Official

FIFRA/Scientific Advisory Panel

DATE:_____