Responses to Major Comments on Technical Support Document

Public Health Goal
For
Molinate
In Drinking Water

Prepared by

Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

June 2008

TABLE OF CONTENTS

INTRODUCTION

The following are the combined responses to major comments received by the Office of Environmental Health Hazard Assessment (OEHHA) on the proposed public health goal (PHG) technical support document for Molinate, based on the pre-release review draft. Changes have already been made in response to these comments, and have been incorporated into the draft. For the sake of brevity, we have selected the more important or representative comments for responses. Comments that are direct quotations appear within quotation marks and paraphrased comments are in italics.

These comments and responses are provided in the spirit of the open dialogue among scientists that is part of the process pursuant to Health and Safety Code Section 57003. For further information about the PHG process, please visit the OEHHA Web site at www.oehha.ca.gov. OEHHA may also be contacted at:

Office of Environmental Health Hazard Assessment
P.O. Box 4010
Sacramento, California 95812-4010
(916) 324-7572

RESPONSES TO MAJOR COMMENTS RECEIVED

Comments from UCLA Reviewer 1

Comment 1: "The PHG is mainly based on a single carcinogenesis study of molinate in rats. I was unable to access the original study (by Pettersen and Richter) on the web. Using the relevant reference cited in the Reference List of the "Goal" brought up a US EPA Cancer Assessment document, rather than the Ciba–Geigy study itself. Some questions do arise, nevertheless, from the summary of this study that is presented in Table 4 of the "Goal". It is stated that only the incidence of combined kidney cortical adenomas and carcinomas (in male rats) was increased, but only at the highest dose. However, did anybody analyze total numbers of tumors at all sites combined? Without seeing the primary data it is not possible to appraise whether the total incidence of tumors increased at lower doses, but the data in Table 4 for those sites that are considered, is suggestive (4, 6, 8, and 19 tumors at 0,7,40 and 300 ppm). I therefore suggest that the data for all sites combined at each dose level should be analyzed."

Response 1: As tempting as it may be to sum all sites, we do not summarize subsets of data that are themselves not significant in order to try to force a significance that could not be suitably supported. In some cases, we do a combined analysis for tumors from different tissues of origin to determine total number of animals bearing tumors related to administration of the chemical, but these data did not support such an analysis.

Comment 2: "I am somewhat concerned also about the calculated "safety level" of 2 ppb for non-cancer endpoints. This level is based on the observed neurological effects of molinate. Molinate reduced the brain weight at all doses tested (as low as 5 ppm) in rats (page 23). Furthermore on page 41 it is reported that there was a decrease in brain weight in rats fed the lowest dose of molinate (300 ppm), although this reduction was considered to be related to body weight changes. In both studies molinate therefore reduced brain weight at the lowest does tested and thus no NOAEL is calculable. It does not therefore seem possible to estimate a non-deleterious dose for the effect of molinate on brain weight."

Response 2: Ordinarily, we prefer to use the highest NOAEL on which to calculate our non-cancer PHG recommendations. However, sometimes it is prudent to use the LOAEL observation in the calculation, such as when no adequate NOAEL exists, or in this case, when the lowest NOAEL is not sufficiently different from the lowest LOAEL. Our procedures permit estimating a NOAEL from the lowest LOAEL by addition of an extra uncertainty factor. We feel that the factor of 10 added in this case for that extrapolation, resulting in a combined uncertainty factor of 1000, results in an adequately health-protective value. In addition, the final PHG is slightly lower than this, because it is based on the carcinogenic endpoint instead of these neurological effects.

Comment 3: "…catfish exposed for 24 hours to 1 ppb molinate were extremely off-flavor (page 7). Although flavor is not a toxic end-point, should we not be concerned that game-fish exposed to the PHG for molinate are essentially inedible?

Response 3: Although the taste of game fish could understandably be a concern for many fishermen, it is not a toxic lesion and is not an indicator of frank toxicity that would be applicable to PHG development. There is a separate process for determining ambient water quality standards, which considers fish consumption.

Comments from UCLA Reviewer 2

Comment 1: "… this reviewer is somewhat concerned about the choice of a 10 fold uncertainty for the use of a LOAEL from the critical non-carcinogenic study, because the critical effect was neurological damage, in contrast to the U.S. EPA's use of a 3 fold uncertainty.

Response 1: It is our opinion that the 10-fold uncertainty factor is generally more prudent when adapting a LOAEL for use in a risk calculation.

Comment 2: "The benchmark dose approach (BMD) may have been of value for assigning a more scientifically based uncertainty value than either the LOAEL used by the U.S. EPA or OEHHA. My criticism is softened by the final C value for the health-protective concentration for carcinogenic effects."

Response 2: We agree that the benchmark dose approach often has utility in risk assessment calculations. We evaluated the Pettersen and Richter (1990) data for both the sciatic and gluteus nerve damage using several of the U.S. EPA BMD models; the data did not lend themselves to an optimal dose-response fit for the BMD models applied. Thus, we chose to base the proposed PHG value recommendation on the standard NOAEL/NOAEL approach. We amended the PHG document to describe this point.

Comment 3: "Molinate at a concentration of 0.5 ug/L causes off-flavor in fish and this presumably renders the flesh inedible. This reviewer can not gage the impact of this on the commercial and sport fishing industry, or on human health and it may in fact be of no consequence. However, final PHG needs to document that these issues have been considered in the risk characterization. The impact of lowered fish consumption due to environmental contaminants is of considerable concern to the public health community. References of recent public debate on the issue are many."

Response 3: The previous commenter stated the same issue. Although the taste of game fish could understandably be a concern for many fishermen, it is not a toxic lesion and is not an indicator of frank toxicity. While interesting, the argument that the flavor might affect fish consumption, and therefore public health, is probably appropriate for development of ambient water quality standards, but not for drinking water standards.

Comments from U.C. Davis Reviewer 1

Comment 1: "Appropriateness: Selection of data sets for risk assessment is always a problem. The set chosen does not seem to have been independently replicated and, like most of the information in the report, it has not appeared in a peer-reviewed publication. Although there is no reason to doubt the experiments themselves, there also is no way to independently evaluate its findings."

Response 1: The commenter is correct that many of the scientific studies available to us were not published in the open literature, thus precluding independent confirmation. Our charge is to use the best quality and most appropriate information available. Particularly for pesticides, this sometimes includes data that are not generally available, but it should be noted that the studies are conducted in accordance with high standards.

Comment 2: "Data evaluation and interpretation: The data seem carefully collected and processed. I am surprised that the evidence that molinate is not only a neurotoxicant, but also a delayed neurotoxicant is not highlighted. There is one study on the chicken in 1983 demonstrating it is a delayed neurotoxicant with similarities in its effects to organophosphates. Evidence is mounting that the mechanism of thiocarbamate neurotoxicity is not only the well-established effects of excess acetylcholine on transmission, but other covalent modifications of cysteine and serine active sites. Recently published work by a long-time investigator of organophosphate induced delayed neuropathy (OPIDN, Lotti, 2002; Moretto et al, 2001) shows that molinate can interact with other neuropathic agents to "promote" their effects. Another recent paper of Zimmerman et al (2004) studied the adducts of molinate and other dithiocarbamates but failed to demonstrate segmental demyelination in peripheral nerves."

Response 2: The evidence that molinate might act as a promoter of delayed organophosphorous neurotoxicity seems relevant to the discussion. We added the following text to the end of the neurotoxicity section:

> Molinate has also been observed to elicit or intensify delayed neuropathy of organophosphorous toxicants (Moretto *et al.*, 2001). In a review of several previous studies, Lotti (2002) included molinate among the esterase inhibitor compounds for which observations of promotion of delayed organophosphorous polyneuropathy have been reported in the chicken. The author distinguishes promotion of delayed neuropathy from novel intoxication by observing that the promoted lesions did not differ from those observed in typical organophosphate induced delayed neuropathy (Lotti, 2002).

Comment 3: "The issue is one that goes beyond fine-tuning the calculated "safe" level of molinate in drinking water, it concerns whether the evidence is sufficient for its deregistration. In other words, would molinate be registered today given the standards for identifying a delayed OPIDN-like neuropathic chemical? Considering the data shown in the Sprague report in 1983, I am surprised that more chicken studies have not been required to verify the findings. Such research should be undertaken if the chemical is not withdrawn from use. The matter is not one of dose-response and establishing NOAELs;

it could be argued that reports that an agent is a delayed neurotoxicant and potentiates neural damage of other agents is sufficient to remove chemicals from use."

Response 3: OEHHA PHGs are risk assessment documents. Whether-or-not it is scientifically justified to remove a chemical from use is a risk management activity, and thus is not within the scope of the document. However, it should be noted that herbicidal uses of molinate have indeed been cancelled.

Comment 4: "Appropriateness of risk assessment methodology: The risk assessment process used in this project is that accepted by the regulatory community. Such a process of choosing one key paper out of many that have been performed, using it to start a risk assessment algebra exercise replete with uncertainty factors to provide safety cushions, should be rigorously re-examined and perhaps replaced with other scientifically and statistically more appropriate methods such as those based on probabilistic risk assessment in which there is a mathematical model where population effects are validated."

Response 4: The process suggested by the reviewer appears worthwhile to pursue in the long term, for development of more comprehensive models and methods.

Comment 5: "Other critical information: The demonstration that the agent affects sperm morphology at low levels clearly demonstrates it is a reproductive toxicant. The low number of studies and sample sizes for chronic exposures for this and other end points is disturbing considering the wide-spread use of the agent. Frankly, the weight of evidence suggests this to be a dirty enough agent to ban instead of arbitrarily choosing low "safe" levels for it."

Response 5: We agree.

Comment 6: "Appropriateness of uncertainties: The issue of using what data are available, bolstering it with uncertainty factors to set acceptable levels is fraught with problems, especially when one considers its impact on the "Risk Cup" approach of the federal EPA in which chemicals with assumed similar modes of action are lumped together to help set a more realistic group exposure. The little evidence available suggests that carbamates like Sevin and molinate may not belong in the same "Risk Cup" based upon what may ultimately be shown to be different modes of toxicity of the agents."

Response 6: We agree that the risk assessment process contains many uncertainties, and the cumulative impact of chemicals with the same mechanism of action is a worthy subject for continued study. U.S. EPA has grouped thiocarbamates together for a cumulative risk assessment for development of tolerances for food crops. In the case of drinking water, coexposures appear to be less common. We believe that in this case, because the PHG is based on carcinogenicity instead of the thiocarbamate enzyme inhibitory effects, that the cumulative (non-cancer) effects are less relevant.

Comments from U.C. Davis Reviewer 2

Comment 1: "A concern throughout the document was the reliance on data from internal company reports that are relatively inaccessible to the general scientific community and abstracts where the data set has not undergone peer review."

Response 1: Yes, many of the scientific studies conducted on this pesticide were not published in the open literature, thus precluding independent confirmation. However, we feel that the rigorous quality control procedures and regulatory oversight for studies submitted for regulatory purposes makes such studies too credible to ignore. Our charge is to use the best quality and most appropriate information available, and often that will be industry-funded studies conducted for regulatory purposes that are not generally available.

Comment 2: "The statement that "the only human data suggestive of dose-dependent metabolism were reported as unpublished results by Krieger et al in Batten et al (1992)" is not completely accurate. Unfortunately, the Batten et al document is again from CTL. The Krieger et al results can be found in abstract form in The Toxicologist. However, it is generally acknowledged that information in abstracts may be preliminary and incomplete."

Response 2: We agree. A cautionary statement has been added to the existing text.

Comment 3: "It should be indicated that the in vitro human data published by Jewell and Miller and described in the document, are also suggestive of dose dependent metabolism."

Response 3: This suggested comment has been added to the metabolism section.

Comment 4: "…the cited publication by Jin and Kitos was only in abstract form and did not supply in depth data."

Response 4: The information was from a presentation at a FASEB conference, and no known full document exists. We agree that this is less substantive than a peer-reviewed publication, and have included it only as supplementary information.

Comment 5: "In the reproductive toxicity section the studies were reviewed and presented in detail. In the study presented on P 22-25, clearly there were many endpoints affected. However, the statement (mid P 23) that "other statistically significant changes in absolute or relative organ weights were not considered to be related to molinate administration" requires some substantiation if a statistical analysis is to be ignored. It seems from the preceding paragraph on this page that molinate affects many organ system both reproductive and nonreproductive."

Response 5: We rephrased this sentence to read "…other statistically significant changes in absolute or relative organ weights were considered by the author to be related either to differences in bodyweight or "isolated occurrences incidental to treatment."

Comment 6: "A significant question is whether the neutral cholesteryl ester hydrolase (nCEH) is inhibited by molinate. It is indicated that this enzyme is responsible for release of cholesterol from HDLs and that molinate specifically blocks this pathway. However, although it has been reported that other esterases are inhibited after administration of molinate it is not clear that nCEH is also inhibited and this effect forms the basis for the proposed hypothesis."

Response 6: The commenter correctly asserts that the proposed mechanism is speculative, and that no clear support exists to demonstrate nCEH inhibition. This is consistent with our text; no change appears necessary.

Comment 7: "In the 2nd paragraph in the same section, it is indicated that molinate sulfoxide would have to be formed in the testis as it is insufficiently stable to circulate in the blood. However, there are literature reports that administration of molinate sulfoxide causes testicular toxicity suggesting that it can reach the testis via the blood, molinate sulfoxide has been measured in the blood, and it is sufficiently stable to be measured in liver microsomal and slice preparations."

Response 7: This sentence was re-worded to remove the statement that molinate sulfoxide is insufficiently stable to reach the testis via blood; the discussion now indicates that the highly reactive intermediate molinate sulfoxide may also be produced in the testes.

Comment 8: "In paragraph 3 in this section, the first section implies that molinate sulfoxide inhibits only an esterase."

Response 8: We do not concur with the commenter's interpretation that the section implies that molinate sulfoxide inhibits only an esterase. No change was made in response to this comment.

Comment 9: "…the study reported by Winder et al is in abstract form and has not undergone peer review."

Response 9: The abstract reported by Winder *et al.* of a presentation at a Society of Toxicology meeting has not been the subject of a formal publication. However, via personal communication with the author, an OEHHA employee, we are assured that none of the data or conclusions have changed.

Comment 10: "Details on neurotoxicity were presented but at least in this section of the document, it was unclear if the same effects had been seen at all dose levels and if there was a dose response relationship for the 3 dose levels (7, 40, 300 ppm)."

Response 10: We added a more precise description of the observations, which generally demonstrated a clear correspondence between dose and response.

Comment 11: "The final dose-response assessment and characterization of Public Health Goals was comprehensively presented although the methods for reaching a Public Health Goal value for carcinogenic effects was not self explanatory. For example, What is a LMS model? Similarly in Risk Characterization, What is a MCLG?"

Response 11: The linear multistage (LMS) model was described in the Carcinogenic Effects section. We have spelled out the confusing acronyms in question.

REFERENCES

Batten PL, Woollen BH, Loftus NJ, Marsh JR, Wilkes MF (1992). Molinate: metabolism in man following a single oral dose. ICI Central Toxicology Laboratory Report No. CTL/R/1099. ICI Americas Inc., Wilmington, DE. March 6, 1992.

Dawson BJM (2001). Shallow ground-water quality beneath rice areas in the Sacramento Valley, California, 1997. U.S. Geological Survey, Water-Resources Investigations Report 01-4000, National Water-Quality Assessment Program, Sacramento, CA.

Domagalski JL, Dileanis PD, Knifong DL, Munday CM, *et al.* (2000). Water-quality assessment of the Sacramento River Basin, California: water-quality, sediment and tissue chemistry, and biological data, 1995-1998. U.S. Geological Survey Open-File Report 00-391. Accessed at: http://water.wr.usgs.gov/sac_nawqa/waterindex.html.

Jin O, Kitos P (1996). Teratogenic synergy between a thiocarbamate herbicide and an organophosphorus insecticide. FASEB J 10(3):A792 [Abstract No. 4576].

Lotti, M (2002). Promotion of organophosphate induced delayed polyneuropathy by certain esterase inhibitors. Toxicology 181-1282: 245-248.

Moretto A, Gardiman G, Panfilo S, Colle MA, Lock EA, Lotti M (2001). Effects of S-ethyl hexahydro-1H-azepine-1-carbothioate (molinate) on di-n-butyl dichlorovinyl phosphate (DBDCVP) neuropathy. Toxicol Sci 62(2):274-9.

Winder BS, Jewell WT, Miller MG (1999). Esterase inhibition by molinate: role in toxicity. Toxicologist 48:382 [Abstract].