State of California

Office of Environmental Health Hazard Assessment

# Memorandum

To : Larry L. Nelson, Ph.D., Chief
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1220 N Street
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Date: September 12, 1991

From : Hazard Identification and
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Subject: Review of Draft Bromoxynil Octanoate Risk Characterization

Please find attached our comments on the draft risk characterization document for bromoxynil octanoate prepared by the Department of Pesticide Regulation (DPR), dated October 3, 1990. We were pleased with the scientific quality of this document. The clarity of the presentation and organization was impressive, even where we disagreed with a chain of logic or a conclusion. Our comments mainly address the limitations of the toxicology data base, the potential for adverse health effects associated with the use of bromoxynil octanoate, and various technical issues.

The major concern about bromoxynil octanoate is that data gaps exist and there is no clear indication of when the situation might be corrected, although the chemical is still registered for use in California. The data gaps, including inadequate studies for combined chronic toxicity and oncogenicity in the rat, chronic toxicity in the dog, reproductive toxicity in the rat, and apparently others, are largely caused by failure of the registrant to demonstrate that the registered product, the octanoate ester of bromoxynil, is toxicologically equivalent to the product tested, the free phenolic form of bromoxynil. The problem is addressed only in the body of the risk characterization document. This also needs to be explicitly articulated in the Executive Summary and the summary.

It is the intention of the Birth Defects Prevention Act that the data gaps on pesticides be filled in order to continue their registration. It is unclear what circumstances mitigate the requirement in this case.

DPR calculations of worker hazard and dermal toxicity are also of concern. This concern would best be met by a comprehensive health risk assessment for agricultural workers based on combined exposure pathways, which could be provided in the revision of this document.

Thank you for the opportunity to review the risk characterization document. If you have any questions concerning this review, please contact me or Dr. Michael DiBartolomeis at 8/571-3063.

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Chief
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Attachment

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## 1.0 GENERAL COMMENTS

Bromoxynil is a selective, postemergence herbicide. The common forms used as pesticides are esters of octanoic and butyric acids. In California in 1989, all formulations containing the butyrate were cancelled. The octanoate ester of bromoxynil is registered for use on wheat, barley, oats, flax, garlic, onions, sorghum, field corn, rye, and various grasses, and also on non-crop areas, such as roadside rights-of-way.

The document submitted for review to the Office of Environmental Health Hazard Assessment is an assessment of the risk of adverse effects on human health associated with bromoxynil octanoate exposure. However, in the majority of the toxicity studies provided to support the registration of bromoxynil octanoate, the unesterified (free phenol) form of bromoxynil was used as a test compound. Although there is evidence that bromoxynil octanoate is at least partly deesterified in the body, it has not been demonstrated that the toxic effects of the octanoate are identical to those of the phenolic form. It is quite possible that absorption, distribution, and the resultant toxicity of bromoxynil octanoate could differ significantly from those of the phenolic form of bromoxynil and its salts.

In Appendix A of the document (Toxicology Summaries), the California Department of Pesticide Regulation (DPR) repeatedly addressed the need to justify testing of the unesterified form to support the registration of bromoxynil octanoate ester. We have no indication that an appropriate justification has been provided by the registrant, although it appears that the unsubstituted and ester forms were considered by DPR to be equivalent for both the exposure and health risk assessments. The 30% molar dose difference between the two forms when doses are expressed in mg/kg appears to have been overlooked. We expect these problems to be corrected in the next version of the risk characterization document.

## 2.0 ADEQUACY OF THE DATA PACKAGE

Toxicological data stated by DPR as currently inadequate or lacking on bromoxynil octanoate include: a combined chronic toxicity/oncogenicity study on rats; a reproductive toxicity study on rats; and a chronic toxicity study on dogs. Previous studies to fill these requirements were judged to be inadequate by DPR partly on the grounds that they were conducted using bromoxynil phenol, not bromoxynil octanoate. Several other studies, including the mouse chronic/oncogenicity study which was judged acceptable by DPR (in a 1985 review), also used the phenolic form. We concur with the judgement of the later (1989) DPR reviews that such studies are not applicable to registration of bromoxynil octanoate without further supporting evidence.

In addition, there are no data available on dermal sensitization (Appendix B, p. 1), which would be highly appropriate for this topical irritant. If it is true that bromoxynil is similar to chemicals such as hydroxyurea in causing delayed eye opening, and to antithyroid compounds in causing neuroanatomic and behavioral changes in neonates, suggesting a potential for developmental neurotoxicity, Office of Environmental Health Hazard Assessment (OEHHA) also recommends neurotoxicity testing.

Although the data on bromoxynil octanoate may be adequate if bromoxynil phenol can be shown to be its toxicological equivalent (see RECOMMENDATIONS), OEHHA concludes that the data are presently inadequate to support its continued registration for use in California.

## 3.0 TOXICOLOGICAL EVALUATION

### 3.1 Subchronic Effects:

DPR described the results of two 13-week oral studies, one on dogs and the other on rats. A dermal toxicity study on rabbits was also discussed in this section. OEHHA agrees with the DPR comment that the studies can only be considered as supplementary. They do not meet all testing requirements, but do contribute useful information. The apparent tolerance of rats to dietary bromoxynil concentrations of 781 and 1953 ppm, for instance, provides supporting evidence that the chronic rat studies may not have used doses up to the maximum tolerated dose (MTD).

### 3.2 Chronic Effects:

The chronic study in mice, with dietary concentrations of bromoxynil phenol up to 118 ppm, shows a modest and significant dose-related increase in combined adenomas and carcinomas of the liver. No evidence was provided that the highest dose studied was equivalent to a maximum tolerated dose (MTD). This may not be considered important because of the significant increase in liver tumors, but failure to use high enough doses can mean that other, rarer tumors would be overlooked. OEHHA agrees with DPR that the single positive study does not provide sufficient evidence to classify bromoxynil as a potential human carcinogen. This study would also be judged unacceptable by the criterion that use of the phenolic form to support registration of the octanoate ester has not been justified.

The two chronic rat studies with bromoxynil fed at up to 600 ppm in the diet provide supplementary information, indicating a probable lack of carcinogenicity in the rat. However, as stated by DPR, justification is required for the selection of doses in these studies because the chronic MTD in the rat was not established. In addition, the use of bromoxynil phenol rather than its octanoate ester was not justified.

A one-year study in dogs with the phenolic form of bromoxynil at up to 7.5 mg/kg-day produced no adverse effects in the animals, but the study suffered from the same problems as above. The study was also judged unacceptable by DPR.

The DPR calculation of toxicity for non-cancer chronic effects (Table 6, page 13) was acceptably health-conservative. Basing this on an extrapolated no effect level for renal hydronephrosis in a chronic rat study yielded an Estimated No Effect Level (ENEL) of 0.5 mg/kg, compared to the lowest No Observed Effect Level (NOEL) among several different studies of 1.5 mg/kg. OEHHA will accept the 0.5 mg/kg value, but believes that the methods for determining the ENEL should be described more clearly in the revised document. In addition, some justification should be provided for making this calculation using a study which DPR has judged to be unacceptable.

It must be noted that <u>all</u> the studies referred to above were or should have been judged unacceptable. If there are good reasons to presume that the studies with bromoxynil are applicable to bromoxynil octanoate, the reasons should be clearly laid out, point by point, in a separate section. Until this situation is resolved, the data on chronic exposure to bromoxynil octanoate do not meet the most basic regulatory requirements.

3.3 <u>Developmental Effects</u>:

Possible adverse effects in reproductive studies on rats included decreased weight gain in weanlings and pups, delayed onset and completion of eye opening, and increased incidence of hydronephrosis and hydroureter in terminated weanlings. Similar effects were obtained in oral and dermal studies. Both the phenolic and the octanoate ester forms of bromoxynil were tested dermally, with comparable results except, apparently, for greater skin irritation with the octanoate.

Because of the opinion expressed by DPR on page 12 of Appendix A that the delayed eye-opening caused by bromoxynil may indicate a potential for developmental neurotoxicity, similar to the effect of hydroxyurea, or that it may act as an antithyroid compound, which could also cause neurotoxicity, DHS recommends neurotoxicity testing.

3.4 <u>Other Effects</u>:

The potential for mutagenicity and other genetic effects was evaluated in a battery of tests, with some positive and some negative results. No clear-cut conclusions can be derived. The limitations of these data should have been summarized. The discussion of the importance of the chemical form of bromoxynil should have mentioned potential differences in transport across

cellular membranes of bromoxynil phenolate and its ester. The esterified form would be non-ionized in solution, and may be much more readily taken up into cells than the phenol, which could potentially result in greater toxicity. Presence of relevant esterases in the rat S9 fraction would be important in this context. In addition, if the octanoate is de-esterified in the stomach and/or intestinal tract before absorption (possible, but not demonstrated), then one would expect that effects could differ by route of administration--i.e., dermal effects might differ from oral effects.

## 4.0 EXPOSURE ASSESSMENT

The DPR draft risk characterization is based on three studies on rats: a dermal teratogenicity study, an oral teratogenicity study, and a reproductive toxicity study. The latter two were judged unacceptable by DPR for multiple reasons; all three would be considered inadequate based on testing of the phenolic form of bromoxynil without demonstration that its effects are equivalent to those of the octanoate ester.

In addition to these problems, OEHHA is concerned about some of the risk assessment procedures and calculations, as explained in the following paragraphs.

### 4.1 Hazard Identification:

Adverse developmental effects were identified in several teratogenicity studies on rats and rabbits. The most sensitive of such endpoints were production of extra ribs and delayed ossification. The lowest NOEL for these effects was 1.5 mg/kg-day of bromoxynil (phenol) in a gavage study on rats, which was judged by OEHHA to be inadequate based on failure to report maternal parameters. Of the available studies, this was judged by DPR to be best for assessing the effects of acute oral exposures to humans.

A two-generation reproductive study on dietary exposure of rats to bromoxynil (phenol) resulted in several effects on the progeny, including a dose-dependent hydronephrosis. The study was judged unacceptable by DPR due to lack of information on several parameters and the failure to justify testing of the free form of the compound. DPR used mathematical modeling to determine an ENEL for hydronephrosis of 0.5 mg/kg-day. This dose was used to estimate human hazard from chronic dietary exposure to bromoxynil, which could be considered health-conservative in that this dose is lower than the NOELs of all the other studies.

A teratogenicity study involving dermal exposure to bromoxynil (phenol) in rats was used by DPR to evaluate risk of developmental effects from occupational exposure to bromoxynil. The NOEL of this unacceptable study, 10 mg/kg-day, was the same as that found in an acceptable rat teratogenicity

study with dermally-administered bromoxynil octanoate. Using a dermal study for estimating dermal risk, instead of the lower ENEL of the oral study discussed in section 3.2, assumes that the apparent difference in toxicity is due to the different routes rather than to the different duration of exposure in the two studies. It is reasonable to assume that a route difference should exist, caused by slow and limited dermal absorption of bromoxynil. However, cumulative effects of bromoxynil may also be relevant. Thus it might not be adequately health-protective to calculate the risk of developmental effects from chronic exposure to bromoxynil in humans based solely on a nine-day teratogenicity study in rats.

OEHHA is concerned that the 20-fold difference in apparent no-effect levels between the teratogenicity studies and the 2-generation reproductive study may overstate the real difference in toxic potential between oral and dermal exposures. This could be considered inconsistent, for example, with the assumed human dermal absorption of bromoxynil of 24% (Appendix B). We recommend that DPR discuss and justify its approach in the revised document.

4.2 Dietary Exposures:

Dietary exposures are calculated based on the tolerance levels. Bromoxynil was reported as not detected in any CDFA analysis of potentially-treated crops in 1989. The potential maximum exposures are shown to be very low in potentially sensitive populations, including children, who are calculated as having over a 600-fold safety margin from the chronic exposure hazard level which is listed above, 0.5 mg/kg-day. However, the listing of a 0.1 mg/kg-day "relevant dose" in food in Appendix C, and a correspondingly lower Margin Of Safety (MOS), provides a potential for confusion. The context of these values should be explained in the revised document.

Although Table 6 on page 13 provides both acute and chronic dietary exposure information, only the acute exposure assessment by the Tolerance Assessment System (TAS) is provided in Appendix C, and exposure value in the appendix is different from that in Table 6. The acute and chronic dietary exposure assessments should be fully supported by TAS exposure calculations in the revised document.

4.3 Occupational Exposures:

The potential exposure of unprotected workers, largely from dermal contact, was shown to be quite high. However, the reasons for the large differences in exposure between the two worker studies described in Appendix B were not clear. Possible effects of the formulations used in these studies should have been addressed, as well as differences in spray equipment and application methods. Also, we had considerable difficulty in following the calculations of dose and risk for dermal exposures. In Tables 6 and 7, applied dose in

rats seems to be compared to absorbed dose in humans in the third columns. If so, the MOS for occupational exposure to bromoxynil would be considerably lower. Possible application of a lower NOEL value for dermal exposures, as discussed in section 4.1, would make MOS still lower.

For subchronic and chronic exposure calculations, average daily exposures could have been corrected for exposures less than seven days per week. Even though the exposures through incidental ingestion, inhalation and diet may be much smaller than dermal exposure, intake through all routes should be explicitly addressed, to provide a combined-route chronic health risk assessment. Total exposure should be compared to the USEPA reference dose (RfD) as well as to the route-specific NOELs derived in this risk assessment.

## 5.0 RISK CHARACTERIZATION

### 5.1 Dietary Exposure:

It appears that the MOS for dietary exposure to bromoxynil octanoate should be adequately health-protective for the general population and sensitive subgroups, considering the calculated exposures of less than 0.001 mg/kg-day. The estimated acute and chronic dietary exposures are also well below the bromoxynil (phenol) RfD of 0.02 mg/kg-day.

### 5.2 Occupational Exposure:

The calculation of the health risk of developmental effects to agricultural workers from occupational exposure is confusing, appearing to compare an administered dose from the animal study to the absorbed dose in humans. If so, the occupational exposure and resulting risk should be recalculated based on equivalent doses.

Risk for both acute and chronic exposures of agricultural workers to bromoxynil octanoate should be determined based on total exposure including occupational dermal exposure and direct ingestion, inhalation, and dietary exposures. The next version of the document should include the scenario for the combined exposures and the resulting MOS.

## 6.0 LABELING/MITIGATION MEASURES

The assumptions as to protection from bromoxynil exposure which can be achieved with various levels of protective equipment should be explicitly discussed and justified. References should be provided, showing why, if penetration studies were carried out with a different chemical or formulation, they should apply to bromoxynil octanoate as currently used. The calculations made in this risk characterization document to show that the proposed risk

mitigation measures (protective clothing, modifications of equipment and work practices) decrease health risk to acceptable levels, have not been substantiated.

## 7.0 SPECIFIC COMMENTS

7.1 Acknowledgements: Change Hazard Evaluation Section to Pesticide and Environmental Toxicology Section.

7.2 Introduction:

- Under usage, bromoxynil butyrate octanoate appears to be a typographical error.

- There are three double bonds missing from the structural formula for bromoxynil octanoate.

7.3 Toxicological Profile:

- Page 3 states that "Bromoxynil octanoate is not a skin or eye irritant". Yet, of the eight reported bromoxynil-related illnesses, two were injuries to skin and three were to the eyes (page 2 and Appendix B page 8). Moreover, Appendix B Page 1 states that bromoxynil dermal exposure caused a erythematic response on rabbit skin which lasted 72 hours, and that bromoxynil is a moderate eye irritant. These discrepancies should be clarified.

- On page 4, it would be useful to the reviewer to know the extent of the mortality in the subchronic rat study at 1953 and 781 ppm. All such summaries should report dose to the animals, not just ppm in their diet. In addition, the duration of the second chronic rat study should have been mentioned.

- The discussions on pages 6 and 7 on whether reported skin irritation caused by dermal application of the commercial product may be due to formulation ingredients would make it appropriate to list those ingredients and their concentrations. Page 2 under Technical and Product Formulations would be a logical place.

- *In vitro* genetic toxicity summaries on page 9 should provide the concentrations of the test chemical in the assay (if calculable), and mention the doses and/or concentrations which caused cytotoxicity, if reported.

7.4 Risk Assessment:

- Table 5, footnote a: We estimate that a dermal dose of 4 mg/rat yields between 10 and 100 ug/cm$^2$, depending on the application area, rather than 0.2 ug/cm$^2$, as claimed. Please correct, or substantiate the DPR estimate. Footnote b: The 50/24 factor in calculation of inhalation dose should be explained.

- Page 12 says that DPR did not find octanoate and butyrate esters of bromoxynil on crops. The several statements in this document that the esters are readily hydrolyzed raise the question of whether this might be an incorrect reporting of the analyses. If only the esters were assayed for on crops, total bromoxynil residues might be greatly underestimated. This should be clarified.

7.5 Quantitative Risk Assessment/Risk Characterization:

- In Table 6, column 1, the choice of exposures should be explained. In column 3, the average absorbed dosage should be stated as ug/kg-day. Also, the calculations of MOS in Tables 6 and 7 should be revaluated or clarified, as discussed above.

7.6 Risk Management:

- On page 14, the statement that there would be a higher concentration of bromoxynil on the skin of occupationally-exposed humans (than was used in rat studies) appears to be incorrect, as discussed above. However, even if true it would not justify the opinion expressed here that this should lead to decreased relative absorption in humans, compared to rats. Such a conclusion requires explanation. On the other hand, it seems quite unlikely that unoccluded dermal absorption in humans would equal occluded dermal absorption in the rat. This could be worth exploring.

- While bromoxynil and its octanoate may be equipotent on a molar basis, there is still a question as to why doses of the two forms (in mg/kg) do not seem to have been converted to one form or the other for consistency in dose and risk calculations. Was everything converted to mg/kg of free bromoxynil, but not stated? Please clarify.

7.7 Appendix B - Occupational Exposure Assessment:

- The molecular formula for bromoxynil octanoate needs another Br.

- With regard to the comment on page 2 that excretory pathways were not [likely to have been] saturated [at a 20 mg/kg dose of bromoxynil], it should be pointed out that very large doses of chemicals are generally required to saturate excretory mechanisms. Although "metabolic pathway"

may have been intended here, ester hydrolysis may also not be readily saturable, depending on the specific mechanism.

- In Table 5 (page 5), the first entry under clothing is "NONE/NONE". If neither long nor short pants and shirts were worn, what were the workers wearing? Also the term "residue convention calculations" is confusing. If it were removed, replaced wholly by the definition which follows, the passage would be clearer.

- Why is the standard body weight assumed to be 54.8 kg (standard woman) in some calculations, but 70 kg (standard man) in others? Do women perform certain tasks (e.g., hand-wand application), whereas men perform others (e.g., mixer/loader)? Either a consistent value should be used, or a rationale for the different values should be provided.

## 8.0 RECOMMENDATIONS

- Clear justification should be provided for testing the free (phenol) form of bromoxynil to support the registration of its octanoate ester. This need could be satisfied by comparative metabolism and distribution studies as suggested by the DPR reviewer. Explicit comparison of the toxicity of the two forms in studies already carried out (such as the two dermal studies discussed in the third paragraph of section 4.2 of this document) would be useful. However, it is also possible that a review of the comparative toxicity of other pesticide acid/ester pairs, combined with information on their enzymatic and non-enzymatic ester hydrolysis rates and physical-chemical properties, might adequately elucidate the chemical and toxicological principles involved.

- The risk characterization should more extensively address uncertainties, including the significance (or lack thereof) of mouse liver tumors, the possibility of neurotoxicity of this chemical, any effects which might justify the wide difference in toxicity which was assumed between oral and dermally applied chemical, and the assumptions used in estimating relative absorption and protection factors.

- If any data gaps remain, they should be clearly addressed, including statements in the summary and the executive summary to the effect that inadequate information has been submitted to adequately address health risk and to fulfill DPR requirements.

- The assessment of health risk for agricultural workers should be revised to include all sources of exposure to bromoxynil, and show the total risk.

- The relative safety of chronic dietary exposure to bromoxynil should be compared with the EPA RfD of 0.02 mg/kg-day as well as with values derived in the DPR risk assessment.

- Other comments should be addressed as indicated.