

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460



Office of Prevention, Pesticides
and
Toxic Substances

MEMORANDUM

October 30, 2001

TXR#: 0050244

SUBJECT: **Molinate - Review of California Rice Commission Submission**

DP Barcode: D278226

Submission: S604229

PC Code: 041402

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Action Required: Review suggested language for the Toxicology Chapter and the Human Health Risk Assessment from the California Rice Commission (CRC).

Recommendation: The CRC's proposed language has been reviewed and considered in this document. At issue were the following: 1) developmental/reproduction characterization; and 2) carcinogen characterization. There has been a recent revision to the molinate cancer classification to "suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential". Therefore, the CRC's suggested revisions are no longer applicable. Regarding the developmental/reproduction issue, RRB1 concludes that: 1) there is evidence of reproduction system effects in multiple species and sexes; 2) there is evidence in the rat prenatal developmental toxicity study and the rat

developmental neurotoxicity study that effects in fetuses/offspring are observed at doses below the maternally toxic dose; and 3) the mechanism of toxicity for reproductive effects proposed only in male rats by the registrant has not been adequately demonstrated. Therefore, the CRC's proposed language revisions to the molinate toxicology chapter are not supported by the currently available data, and RRB1 cannot accept the suggested revisions concerning the reproductive/developmental effects of molinate.

BACKGROUND

Two human health risk assessments, dated June 15, 2000 and January 9, 2001, have been forwarded to SRRD from HED. It appears that the California Rice Commission (CRC) is suggesting revised language based on the original document. One of the revisions to the original document involved the cancer reassessment of molinate by the HED Cancer Assessment Review Committee (CARC). The basis for this reassessment was the submission of new data and a reanalysis of the kidney tumor data. The CARC classified the data for molinate into the category "suggestive evidence for carcinogenicity but not sufficient to assess human carcinogenic potential" based on the limited evidence of kidney tumors in rats. The Committee further concluded that quantification of carcinogenic risk is not required.¹

REVIEW

The CRC has submitted suggested language for the Molinate Toxicology Chapter and the Human Health Risk Assessment that they believe will "... enable the Agency to fulfill its obligations under FQPA while minimizing the risk of triggering a Proposition 65 listing in California." The language suggestions deal with: 1) developmental/reproductive toxicity characterization issue; and 2) carcinogen characterization issue.

1. Developmental/Reproductive Toxicity Characterization Issue

The CRC is concerned that the current language in the Reregistration Eligibility Decision (RED) documents overstates the potential reproductive/developmental toxicity of molinate and downplays the new mechanistic data that "strongly suggests" that the effects observed in rodents are not relevant to humans. The CRC believes that the Agency's conclusions will cause the State of California Office of Environmental Health Hazard Assessment (OEHHA) to list molinate as a known reproductive/developmental toxicant under Proposition 65 and thus deny the rice growers of California the use of molinate. The CRC's rationale for the suggested language changes are the following:

- there is no scientific justification for the broad general statement that molinate is a reproductive toxicant
- molinate causes adverse reproductive effects only in male rats; the statement that molinate causes adverse reproductive effects across species (rat, dog, monkey and rabbit) is without support
- there is no developmental toxicity at levels below those causing maternal toxicity in any species
- a growing body of evidence supporting the proposition that adverse effects in rodents are rodent-specific has been ignored

¹Molinate - Report of the Cancer Risk Assessment Review Committee dated December 14, 2000.

Supporting information on this issue include the following:

- there is no evidence that molinate is a developmental toxicant in the rat or rabbit; any effects seen in the developing fetus was at a maternally toxic dose
- adverse reproductive effects are limited to male rats; the reported high-dose effects on sperm morphology (abnormal and atypically stained heads) in rabbits observed by light microscopy are slight were slight, distinct from effects seen in the rat, of unknown biological significance and could not be confirmed by scanning electron microscopy
- the variability in pre-implantation loss in rabbits combined with flaws in study design make an attempt to ascribe an increase to molinate scientifically unjustifiable
- in the dog study, even at the high dose of 100 mg/kg/day where frank toxicity was observed, there was no evidence that molinate causes adverse reproductive effects
- in cynomolgus monkeys, no adverse reproductive effects were seen at dose levels of up to and including 50 mg/kg/day, a dose which caused significant blood cholinesterase inhibition
- there is a significant and growing body of mechanistic data generated by the registrant and others that strongly supports the view that the adverse reproductive effects observed in rodents are not relevant to non-rodents, including humans

Suggested Changes in Wording of RED Documents

The CRC has submitted suggested wording for both the Toxicology Chapter and the Human Health Risk Assessment. As the wording is similar, only the changes to the Human Health Risk Assessment of the RED will be discussed in this review. The following changes are suggested:

A) Executive Summary

The CRC suggests the deletion of the sentence, "The findings in multiple studies demonstrate that molinate is both a neurotoxin and a reproductive toxicant after single and multiple doses via the oral, dermal and inhalation routes of exposure and across species (rat, dog, mouse, monkey and rabbit)." It should be noted that in the Revised Human Health Risk Assessment dated January 9, 2001, the monkey is not included in the list of species.

B) Page 5

The CRC would delete the following sentences (strike-out) and substitute the language in italics.

In the developmental neurotoxicity study, pups born to molinate-treated dams exhibited treatment-related functional and anatomical nervous system effects. ~~Evidence of reproductive toxicity was found~~

~~in studies in rats, mice, and dogs; however, the male rat appeared to be the most sensitive species/sex. A wide range of male reproductive parameters have been altered adversely in the studies with both oral and inhalation exposures, including testes weight, sperm number and morphology, fertility and testicular histopathology. Reproduction studies in both rats and mice demonstrated treatment-related effects on fertility and gestation. In a special five-week fertility study, a dose of 0.5 mg/kg/day in males produced adverse effects on sperm parameters; a NOAEL was not established. There was also evidence that molinate causes increased sensitivity to offspring following prenatal exposure in rats.~~

Molinate is generally considered to be a reproductive toxicant in male rats. Typical effects seen include abnormal sperm, decreased sperm motility and reduced sperm counts. Any effect in female rats is less clear. The few adverse effects reporting (sic) in other species (dog, monkey and rabbit) are equivocal and are not consistent with the effects seen in the rat. Further, recent mechanistic studies done by the registrant and others call into question the relevance of the effects seen in the rat to other species, including humans.

Delayed fetal development was observed in the rabbit (delayed ossification) and the rat (runting) at the same dose level where maternal toxicity was observed. Some uncertainty exists regarding the runting seen in the rat since the original DER (EPA 1990) concluded the effect occurred only at maternally toxic doses. Subsequent to that time, other reviewers have confirmed this conclusion (CDPR 1998). In light of this, the study must be reevaluated relative to biological significance and maternal toxicity.

In the rat developmental neurotoxicity study a reduction in startle response was noted at doses below those causing maternal toxicity. However, the effect was transient in nature, was observed in only one sex and one species and is of questionable biological relevance.

C) Page 6

The CRC would delete the following sentences and substitute the language in italics.

~~The 10x FQPA Safety Factor has been retained based on the following: increased fetal susceptibility observed in the prenatal developmental study in rats; increased fetal susceptibility in the developmental neurotoxicity study in rats; reproductive effects in mice and rats; and uncertainty associated with the molinate surface water exposure in some rice-growing areas~~ *recommendation of the FQPA Safety Factor Committee.*

D) Page 10

The suggested changes from the CRC in strikeout and italics for the following paragraph include:

~~Delayed fetal development was observed in the rabbit at the same dose level where maternal toxicity was observed. In the rat, developmental toxicity/developmental neurotoxicity were observed [increase in runting/reduction in startle amplitude] at dose levels below the maternal NOAEL. Molinate is a~~

~~reproductive toxicant, and the rat is the most sensitive species for this effect. Abnormal sperm, decreased percent motile sperm, decreased sperm numbers, decreased litter size, decreased percentage of pups born live, decreased pup viability, increased incidence of microscopic lesions in the ovary, testes, and adrenal, delayed vaginal opening, reproductive organ weight effects, and decreased brain weight were consistent findings in studies in the rat. In a special five-week fertility study, a dose of 0.5 mg/kg/day in males produced adverse effects on sperm parameters; a NOAEL was not established. Molinate is generally considered to be a reproductive toxicant in male rats. Typical effects seen include abnormal sperm, decreased sperm motility and reduced sperm counts. Any effect in female rats is less clear. The few adverse effects reporting (sic) in other species (dog, monkey and rabbit) are equivocal and are not consistent with the effects seen in the rat. Further, recent mechanistic studies done by the registrant and others call into question the relevance of the effects seen in the rat to other species, including humans.~~

Delayed fetal development was observed in the rabbit (delayed ossification) and the rat (runting) at the same dose level where maternal toxicity was observed. Some uncertainty exists regarding the runting seen in the rat since the original DER (EPA 1990) concluded the effect occurred only at maternally toxic doses. Subsequent to that time, other reviewers have confirmed this conclusion (CDPR 1998). In light of this, the study must be reevaluated relative to biological significance and maternal toxicity.

In the rat developmental neurotoxicity study a reduction in startle response was noted at doses below those causing maternal toxicity. However, the effect was transient in nature, was observed in only one sex and one species and is of questionable biological relevance.

It is the registrant's position that the reproductive effect of molinate "requires the production of molinate sulfoxide and the dependence on the enzyme cholesterol ester hydrolase (CEH) for steroid sex hormone production." Additionally, the registrant concludes that the reproductive toxicity in the rat is induced by a mechanism that is specific to rodents. Special studies data submitted to establish the proposed mechanism of toxicity were reviewed and evaluated by the HED Mechanism of Toxicity Assessment Review Committee. *The studies raise significant questions regarding the relevance of the effects seen in the rat to other species, including humans.* ~~The~~ However, the Committee concluded that the submitted studies are not yet ~~adequate~~ sufficient to demonstrate the proposed mechanism of toxicity. The details of the reasons for the Committee's conclusions are included in the memorandum of that meeting. ~~Some of the reasons include the following: lack of concordance between dose levels where effects on testosterone and precursor hormone levels are observed and dose levels where fertility/sperm effects are observed; lack of data to show that sulfoxidation is occurring at the dose levels where fertility/sperm effects are observed in the rat; and lack of data demonstrating an inhibition of n-CEH in vivo at dose levels where fertility/sperm effects occur.~~

D) Page 11, 3.2 FQPA Considerations

The suggested changes from the CRC are in strikeout and italics.

There is evidence of neurotoxicity in multiple studies with several species. ~~Increased susceptibility of Neurotoxic effects [reduced startle amplitude] in offspring was were observed in the prenatal developmental toxicity study and the developmental neurotoxicity study in rats.~~ The HED FQPA Safety Factor Committee evaluated the hazard and exposure data for molinate as the bases for making a recommendation on the magnitude of the FQPA Safety Factor. The FQPA Safety Factor Committee recommendation in the December 17, 1998 report of the October 30, 1998 meeting was that the **FQPA Safety Factor be retained at 10X for molinate.** ~~The rationale for the retention of the 10X is:~~

- ~~• Increased susceptibility observed in the prenatal developmental toxicity study in rats.~~
- ~~• Increased susceptibility observed in the developmental neurotoxicity study in rats.~~
- ~~• Reproductive effects were seen in mice (anti-fertility study) and rats (sperm morphology study) following oral administration (although there was no evidence of increased susceptibility in the 2-generation reproduction study).~~
- ~~• Uncertainty associated with the lack of characterization for the surface water monitoring data used for drinking water exposure assessments. The environmental fate data base indicates that the parent molinate is persistent and expected to reach surface water. Monitoring data are available, however there is a lack of characterization of the exposure levels for localities downstream of rice fields in the Southeast.~~

The Committee determined that the 10x FQPA safety factor is applicable for following: the *acute dietary risk assessment and the chronic dietary risk assessment.*

Acute Dietary Assessment: ~~The Committee determined that the FQPA Safety Factor should be retained (10x) for acute dietary risk assessment because the increased susceptibility was demonstrated in both the prenatal developmental toxicity and developmental neurotoxicity studies.~~

Chronic Dietary Assessment: ~~The Committee determined that the FQPA Safety Factor should be retained (10x) for chronic dietary risk assessment because of the concern for the severe reproductive effects seen following repeated oral exposures in studies with rats and mice.~~

2. Carcinogen Characterization Issue

The arguments made on this issue involve the classification of molinate as a Group C carcinogen and the use of a Q_1^* for risk quantification. As discussed under Background, the CARC classified the data for molinate into the category "suggestive evidence for carcinogenicity but not sufficient to assess human carcinogenic potential" based on the limited evidence of kidney tumors in rats. The Committee further concluded that quantification of carcinogenic risk is not required. Therefore, the CRC's arguments are no longer applicable and will not be discussed in this review.

DISCUSSION/CONCLUSIONS

The CRC's proposed revisions will be discussed as they appear in the review above.

A) Executive Summary

The CRC thinks the statement that molinate causes reproductive effects across species is without support. RRB1 concludes that there is evidence of reproductive system effects in both sexes and multiple species. In addition, the HED assessment is consistent with that of the California Department of Pesticide Regulation (CDPR) Health Assessment dated March 3, 2000 for molinate. CDPR concluded that reproductive effects were observed in male rats, mice and rabbits treated directly with molinate, in female rats, mice and rabbits mated with treated males and in female rats and mice treated directly.

It is generally accepted that molinate is a reproductive toxicant in male rats. Numerous studies in female rats demonstrated ovarian effects (increased interstitial vacuolation/hypertrophy). In a modified reproduction study in which treated females were mated with untreated males, there was a decrease in: 1) number of uterine implants; 2) litter size; 3) fertility index; and 4) gestation index.

There are also data in female mice demonstrating reproductive system effects. In the mouse carcinogenicity study, there was an increase in thecal/interstitial cell hyperplasia in the ovaries at the highest two doses. At the highest dose, there was also atrophy of the uterus and mammary gland.

Numerous nonguideline studies were conducted to explore the effects of molinate on the reproductive systems of rabbits; however, there was significant mortality due to excessive doses in many studies. The studies consistently show a decrease in the mean number of live fetuses at the highest dose level tested (one not causing deaths) in each study. An increase in sperm abnormalities (atypically stained sperm heads, mid-piece abnormalities) was also a consistent finding in these studies. In the rabbit prenatal developmental study, there was a decrease in the percentage of does with live fetuses and an increase in abortions at the high dose.

The data in the dog are limited as only one study (chronic toxicity) is available in the toxicology database. There was a decrease in ejaculate volume and percentage motile sperm at the 50 mg/kg/day dose level (4 doses: 1, 10, 50, 100 mg/kg/day). The high dose was terminated after 14 weeks [empty capsules thereafter] due to excessive toxicity. Sperm analyses performed at 6 months and 12 months. At the 6-month interval, the % motile sperm at 50 and 100 mg/kg/day was 47% and 49%, respectively, compared to 62% in control; % abnormal sperm was 25% and 32%, respectively compared to 21% in control; % live sperm was 50% at 100 mg/kg/day vs 66% in control and 67% in 50 mg/kg/day group.

B) Page 5

The CRC states that mechanistic studies call into question the relevance of the effects seen in the rat and other species, including humans. These data were peer reviewed by the HED Mechanism of Toxicity Assessment Review Committee on January 13, 2000 and found to be inadequate to demonstrate the proposed mechanism of toxicity. The CDPR also concluded that the mechanistic studies do not fully support the argument for rodent specificity.

The CRC disagrees with HED's conclusions for the rat prenatal developmental toxicity study (MRID 41473401) and the rat developmental neurotoxicity study (MRID 44079201) in which fetal/offspring effects were observed at dose levels below the maternally toxic dose. Regarding the rat developmental study, the CRC's proposed language states that there is some uncertainty about the runting effect in the rat since the original DER (1990) concluded the effect was only seen at maternally toxic doses. In the original DER, the maternal and developmental toxicity NOAEL was set at 35 mg/kg/day. The study was reevaluated in 1992 by the HED Reproduction and Developmental Toxicity Committee and the DER was revised to set the developmental toxicity NOAEL at 2.2 mg/kg/day based on an increase in runting at 35 mg/kg/day. The maternal NOAEL was retained at 35 mg/kg/day. Since then, this conclusion has been peer reviewed and accepted by both the Hazard Identification Assessment Review Committee (HIARC) and the FQPA Safety Factor Committee. No new data have been submitted since these 1998 meetings. Therefore, RRB1 cannot accept CRC's proposed language that there is uncertainty about the runting effect and the study must be reevaluated.

The CRC proposed revision on the rat developmental neurotoxicity study states that the reduction in startle response is of questionable biological relevance as it was transient and observed in only one sex and species. RRB1 does not agree with this statement. In this study, the maternal NOAEL was 75 ppm (6.9 mg/kg/day) based on decreased body weight, body weight gain and food consumption at 300 ppm (26.1 mg/kg/day). There was no developmental neurotoxicity NOAEL based on increased startle amplitude in the auditory startle test in female offspring on day 23 at 20 ppm (1.8 mg/kg/day), the lowest dose tested. It should be noted that this is the only study in the toxicology database in which neurological parameters were tested in offspring. HED's conclusions for this study were also peer reviewed and accepted by both the Hazard Identification Assessment Review Committee (HIARC) and the FQPA Safety Factor Committee. No new data have been submitted since these 1998 meetings. CDPR established the maternal NOAEL at the same dose but set the developmental neurotoxicity NOAEL at 2 mg/kg/day (rounded off from 1.8 mg/kg/day) based on reduced thickness of the molecular layer of the prepyramidal fissure of the cerebellum (day 12) at 7.5 mg/kg/day (difference in calculating mg/kg/day dose at 75 ppm). Although there was a difference in NOAEL/LOAEL, CDPR also determined that offspring developmental neurotoxicity effects were observed at doses below the maternally toxic dose.

C) Page 6

The CRC suggested language would be just a statement without supporting information, and it would essentially eliminate the basis for setting a 10x FQPA Safety Factor for molinate. RRB1 thinks the findings of the FQPA Safety Factor Committee should be included.

D) Page 10

The CRC's position on the reproductive and developmental effects have been addressed under B).

The CRC suggests adding that the reproduction mechanistic studies raise significant questions about the relevance of effects in rats relative to other species, including humans. The next sentence states that the Mechanism of Toxicity Committee concluded that the submitted studies are not yet sufficient to demonstrate the mechanism of toxicity. As discussed above, the Committee concluded that the data were not adequate to establish a mechanism of the reproductive toxicity in male rats only. In addition, there is evidence in the toxicology database that reproductive effects are observed in female animals and in other species that has not been satisfactorily addressed by the registrant. Therefore, the available data are not sufficient in demonstrating the reproductive effects of molinate are only relevant to male rats.