MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Molinate

FROM: Linda L. Taylor, Ph.D.
Toxicology Branch II, Section II,
Health Effects Division (H7509C)

and

Esther Rinde, *Ph.D.
Manager, Carcinogenicity Peer Review Committee
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

TO: Robert Taylor
Pesticide-Herbicide Branch
Registration Division (H7505C)

and

Jay Ellenberger, Chief
Accelerated Reregistration Branch
Special Review and Reregistration Division (H7508W)

The Health Effects Division Carcinogenicity Peer Review Committee met on 6/17/92 to discuss and evaluate the weight-of-the-evidence on molinate with particular reference to its carcinogenic potential.

The Peer Review Committee agreed that molinate should be classified as Group C - possible human carcinogen and recommended that for the purpose of risk characterization, a low dose extrapolation model applied to the experimental animal tumor data should be used for quantification of human risk (q.)

A. Individuals in Attendance:

1. **Peer Review Committee:** (Signatures indicate concurrence with the peer review unless otherwise stated.)

Karl Baetcke

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<table>
<thead>
<tr>
<th>TOXIC CHEM. NO.</th>
<th>ETNFR:</th>
<th>CURRENT DATE</th>
<th>FEMA ACCESS</th>
<th>MATERIAL</th>
<th>CITATION</th>
<th>CARCINOGENICITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-115-101-9</td>
<td>009760</td>
<td>09/17/92</td>
<td>121099</td>
<td>Mcllvaite</td>
<td>McIlvaine</td>
<td>C(*)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>009761</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2. **Reviewers:** (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

- Linda L. Taylor
- K. Clark Swentzel
- Bernice Fisher
- Lori Brunsman

3. **Peer Review Members in Absentia:** (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

- Penelope Fenner-Crisp
- Richard Hill
- Reto Engler
- Julie Du
- George Ghali
- Lucas Brennecke
- Jean Parker
- John Quest

\[1\text{Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.}\]
4. Other Attendees:

Eve Andersen (Clement)
Robert Kovatch (PAI, attended for Lucas Brennecke)

B. Material Reviewed:

The material available for review consisted of DER’s, one-liners, and other data summaries prepared by Linda L. Taylor; tables and statistical analysis by Lori L. Brunsman. The material reviewed is attached to the file copy of this report. The data reviewed are based on studies submitted to the Agency by ICI Americas, Inc.

C. Background Information:

Molinate [S-ethyl hexahydro-1H-azepine-1-carbothioate] is a selective, pre-emergence, thiocarbamate herbicide for use on rice only. It is known by the tradename Ordram® and R-4572. The molecular formula is $C_8H_{17}NOS$ (MW 187.3). Molinate has a vapor pressure of $5.5 \times 10^{-3}$ mm Hg at 25°C and a solubility of 880 mg/L in water at 20°C.

The Caswell (or Tox Chem) Number of molinate is 444.
The Chemical Abstracts Registry Number (CAS No.) is 2212-67-1.

The structure of molinate is:

```
\begin{center}
\text{O} \\
\text{C} \\
\text{C} \\
\text{C} \\
\text{C} \\
\text{C} \\
\text{C} \\
\text{C} \\
\text{C} \\
\text{C} \\
\text{C} \\
\end{center}
```
D. Evaluation of Carcinogenicity Evidence:

1. Rat Carcinogenicity Study

Reference: Pettersen, JC and Richter, AG. Two-Year Chronic Toxicity/Oncogenicity Study with R-4572 in Rats. Study # T-13023, Ciba-Geigy Corporation/Environmental Health Center; ICI Americas Inc. MRID # 418151-01

a. Experimental Design

R-4572 was administered by dietary admix to 50 Cr1:CD®(SD)®R rats/sex/group for two years at dose levels of 0, 7, 40, or 300 ppm; 600 ppm was administered for one year only to a satellite toxicity group (20 rats/sex) for "evaluation of pathology other than neoplasia." There was a 12-month interim sacrifice of 20 rats/sex (control) and 10 rats/sex (treated).

b. Discussion of Tumor Data

The tumor data, with statistical analysis, in male rats is shown in Tables 1, 2, and 3. Females had no significant increases in numbers of tumors at any site. Because there was less mortality at the high dose, Peto's prevalence test was used where there were significant tumors at the designated times and dose levels. If there were insufficient tumor data, the Exact trend test and Fisher's Exact test were utilized.

Kidneys

In male rats there was a statistically significant increase by pair-wise comparison of controls with the 300 ppm group for combined kidney cortical adenomas and/or carcinomas (p<0.05). Male rats also had significant positive trends for kidney carcinomas (p<0.05), and combined kidney adenomas and/or carcinomas (p<0.01).

There are no data available to suggest that the kidney tumors are associated with alpha-2u-globulin for this chemical.

Historical control data for the kidney tumors showed adenomas ranging from 0 (5 studies) to 3.3% (one study) mean of 0.93%; kidney carcinomas showed a range of 0 (7 studies) to 3.3% (one study) mean of 0.56%. The PRC noted that the historical control data came from the years 1980-1986, while the study was performed in 1989-1991. The historical data analysis was performed using non-censored data, while the analysis of the present study utilized censored data. The PRC determined that the kidney tumors in the present study were present at numbers above the mean and range for historical controls. The kidney tumors in male rats are considered to be a rare tumor type.
Table 1. Molinate - Charles River Crl:CD(SD)BR Rat Study

Male Kidney Tumor Rates* and Exact Trend Test and Fisher's Exact Test Results (p values)

<table>
<thead>
<tr>
<th>Tumors:</th>
<th>0</th>
<th>7</th>
<th>40</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortical Adenomas</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%)</td>
<td>0/57</td>
<td>0/46</td>
<td>0/49</td>
<td>2⁰/48</td>
</tr>
<tr>
<td></td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(4)</td>
</tr>
<tr>
<td><strong>p =</strong></td>
<td>0.057</td>
<td>1.000</td>
<td>1.000</td>
<td>0.207</td>
</tr>
<tr>
<td><strong>Carcinomas</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%)</td>
<td>0/57</td>
<td>0/46</td>
<td>0/49</td>
<td>3²/48</td>
</tr>
<tr>
<td></td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(6)</td>
</tr>
<tr>
<td><strong>p =</strong></td>
<td>0.013*</td>
<td>1.000</td>
<td>1.000</td>
<td>0.092</td>
</tr>
<tr>
<td><strong>Combined</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%)</td>
<td>0/57</td>
<td>0/46</td>
<td>0/49</td>
<td>5/48</td>
</tr>
<tr>
<td></td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(10)</td>
</tr>
<tr>
<td><strong>p =</strong></td>
<td>0.001**</td>
<td>1.000</td>
<td>1.000</td>
<td>0.018*</td>
</tr>
</tbody>
</table>

*Number of tumor bearing animals/Number of animals examined, excluding those that died before week 55.

*First adenoma observed at week 106, dose 300 ppm.

²First carcinoma observed at week 92, dose 300 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then p < 0.05. If **, then p < 0.01.
Table 2. Molinate - Charles River Crl:CD(SD)BR Rat Study

Male Hepatocellular Tumor Rates* and
Peto Prevalence Test Results (p values)

<table>
<thead>
<tr>
<th>Dose (ppm)</th>
<th>0</th>
<th>7</th>
<th>40</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumors:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenomas (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/40</td>
<td>1/31</td>
<td>1* /36</td>
<td>3/45</td>
</tr>
<tr>
<td></td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(7)</td>
</tr>
<tr>
<td>p =</td>
<td>0.148</td>
<td>0.574</td>
<td>0.391</td>
<td>0.218</td>
</tr>
<tr>
<td>Carcinomas (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0/40</td>
<td>0/33</td>
<td>2b /37</td>
<td>2/46</td>
</tr>
<tr>
<td></td>
<td>(0)</td>
<td>(0)</td>
<td>(5)</td>
<td>(4)</td>
</tr>
<tr>
<td>p =</td>
<td>0.100</td>
<td>-</td>
<td>0.053</td>
<td>0.088</td>
</tr>
<tr>
<td>Combined (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/40</td>
<td>1/33</td>
<td>3/37</td>
<td>5/46</td>
</tr>
<tr>
<td></td>
<td>(3)</td>
<td>(3)</td>
<td>(8)</td>
<td>(11)</td>
</tr>
<tr>
<td>p =</td>
<td>0.047</td>
<td>0.594</td>
<td>0.082</td>
<td>0.071</td>
</tr>
</tbody>
</table>

*Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

*First adenoma observed at week 87, dose 40 ppm.

bFirst carcinoma observed at week 86, dose 40 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then p < 0.05. If **, then p < 0.01.
Table 3. Molinate - Charles River Crl:CD(SD)BR Rat Study

**Male Testicular Tumor Rates** and **Peto Prevalence Test Results (p values)**

<table>
<thead>
<tr>
<th>Tumors:</th>
<th>0</th>
<th>7</th>
<th>40</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Interstitial</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell Tumor (%)</td>
<td>3/45</td>
<td>5/40</td>
<td>5½/42</td>
<td>7/48</td>
</tr>
<tr>
<td>p =</td>
<td>0.280</td>
<td>0.219</td>
<td>0.222</td>
<td>0.167</td>
</tr>
<tr>
<td><strong>Mesothelioma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%)</td>
<td>0/41</td>
<td>0/35</td>
<td>0/40</td>
<td>2½/47</td>
</tr>
<tr>
<td>p =</td>
<td>0.013*</td>
<td>-</td>
<td>-</td>
<td>0.089</td>
</tr>
</tbody>
</table>

*Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

First interstitial cell tumor observed at week 77, dose 40 ppm.

First mesothelioma observed at week 83, dose 300 ppm.

**Note:** Significance of trend denoted at **control**.

Significance of pair-wise comparison with control denoted at **dose** level.

If *, then p < 0.05. If ***, then p < 0.01.
Liver
In male rats there was a statistically significant positive trend for combined hepatocellular adenomas and/or carcinomas (p<0.05), but no statistically significant trend or increase by pair-wise comparisons for adenomas or carcinomas alone. The PRC determined that this was suggestive evidence, but not sufficient evidence to conclude that molinate administration was responsible for these liver tumors. The numbers of liver tumors were considered to be within historical controls.

Testes
The PRC discussed the fact that since interstitial and mesothelial tumors in the testes originate from different cell lines, they should not be combined. These tumors tend to be malignant.

There was a significant trend for testes mesotheliomas (p<0.05) with increasing dose levels of molinate. Historical control data on testes mesothelioma tumors were not available to the PRC.

There was no statistically significant increase in interstitial cell tumors, but the PRC noted that in the high dose group (300 ppm) the interstitial cell tumors of the testes occurred (7/48, 15%) at twice the rate of controls (3/45, 7%). Of the 20 male rats fed 600 ppm molinate for 12 months, there was one tumor (testes interstitial cell tumor) found in one animal only. Testicular interstitial cell tumors were reported as ranging from 0 (2 studies) to 6.7% (1 study); mean of 2.98%.

The PRC determined that there is insufficient evidence to show that the formation of testicular mesotheliomas is associated with the administration of molinate. However, non-neoplastic lesions
of the testes and adverse reproductive effects lend support to
the contention that testes tumors are of concern.

Also, the Registrant informed the Agency previously of a 1980
study in which an increase in the incidence of interstitial cell
tumors was observed. It was stated that the raw data from that
study were not retained (Japanese), and the study could not be
verified. The dose levels and incidence of testicular tumors in
that study are shown below.

Testicular Tumor Incidence

<table>
<thead>
<tr>
<th>Dose (ppm)</th>
<th>0</th>
<th>5</th>
<th>50</th>
<th>100</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td># rats examined</td>
<td>48</td>
<td>45</td>
<td>46</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>Interstitial Cell Tumors (intercurrent)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Interstitial Cell Tumors (terminal)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Combined</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>11</td>
</tr>
</tbody>
</table>

c. Non-neoplastic Lesions

Lesions in the reproductive organs were as follows:

<table>
<thead>
<tr>
<th>Parameter/ppm</th>
<th>0</th>
<th>7</th>
<th>40</th>
<th>300</th>
<th>600 (1 year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TESTES: degeneration w/ atrophy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>grades 182</td>
<td>70</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>grade 3</td>
<td>9</td>
<td>7</td>
<td>3</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>grades 485</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>OVARIES: thecal/interstitial cell vacuolation/ hypertrophy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>grades 182</td>
<td>69</td>
<td>60</td>
<td>60</td>
<td>58</td>
<td>20</td>
</tr>
<tr>
<td>grade 3</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>grades 485</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EPIDIDYMIDES: atrophy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>grades 182</td>
<td>70</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>grade 3</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>grades 485</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>oligospermia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>grades 182</td>
<td>9</td>
<td>7</td>
<td>7</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>grade 3</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>grades 485</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Degeneration/demyelination of the sciatic nerve was increased in incidence and severity with increasing dose in both sexes. The incidence of skeletal muscle (thigh) atrophy/reserve cell hyperplasia was increased with increasing dose in both sexes, and the males also displayed increased severity with increasing dose compared to the controls. In the spinal cord, the incidence of degeneration and/or eosinophilic bodies was increased in both sexes with increasing dose, especially in the sacral region.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The dosing was considered to be adequate for assessing the carcinogenic potential of molinate in rats. Body weight was decreased slightly throughout the study in both sexes at the 300 ppm dose level compared to control values, and body-weight gain was decreased throughout the study in both sexes at this dose level compared to the control values. At 90 days, body-weight gain was 83\% of the control value in the males and 83\% of the control value in females of the 300 ppm dose group.

<table>
<thead>
<tr>
<th>(weeks)</th>
<th>MALES</th>
<th></th>
<th>FEMALES</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 ppm</td>
<td>40 ppm</td>
<td>300 ppm</td>
<td>7 ppm</td>
</tr>
<tr>
<td>0-4</td>
<td>96</td>
<td>90</td>
<td>87</td>
<td>96</td>
</tr>
<tr>
<td>0-13</td>
<td>98</td>
<td>93</td>
<td>85**</td>
<td>95</td>
</tr>
<tr>
<td>0-26</td>
<td>97</td>
<td>92*</td>
<td>85**</td>
<td>93</td>
</tr>
<tr>
<td>0-50</td>
<td>99</td>
<td>96</td>
<td>85**</td>
<td>96</td>
</tr>
<tr>
<td>0-78</td>
<td>100</td>
<td>94</td>
<td>91</td>
<td>99</td>
</tr>
<tr>
<td>0-104/5</td>
<td>91</td>
<td>100</td>
<td>79**</td>
<td>105</td>
</tr>
</tbody>
</table>

* p<0.05
** p<0.01

At the 12-month sacrifice, a dose-related decrease in brain weight was observed in both sexes, with statistical significance being attained at 300 ppm in males and at 600 ppm in both sexes. Brain weight was decreased also at the 2-year (final) sacrifice in both sexes at the highest dose (300 ppm) level. Other organ weight changes were observed at the 300 and 600 ppm dose levels only and can be attributed to decreased body weight. Red blood cell cholinesterase was decreased in both sexes at the two highest dose levels at various times during the study, although statistical significance was not always attained.
Survival was not adversely affected by treatment. At study termination (24 months), the high-dose group displayed the highest survival rate for both sexes.

2. Mouse Carcinogenicity Study


a. Experimental Design

Molinate was fed to Crl:CD-1 (ICR) BR mice (50/sex/group) at dose levels of 0, 10, 100, 1000, or 2000 ppm for 18 months.

b. Discussion of Tumor Data

There was no treatment-related effect on the incidence of any neoplastic lesion. Those lesions observed in the study were of the type common to the CD-1 mouse.

c. Non-neoplastic Lesions

Several clinical observations indicative of neurological involvement were observed with higher incidence at the 2000 ppm dose level in both sexes, which were accompanied by/correlated with nonneoplastic lesions (increased incidence of demyelination and Schwann cell hyperplasia of the sciatic nerve, presence of eosinophilic bodies at the cervical/lumbar/sacral/thoracic levels of the spinal cord and in the brain). Additionally, there was an increase in the incidence of degeneration of the testes (dose-related) and in the incidence of ovarian hyperplasia (thecal/interstitial cell).

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The PRC determined that the dosing was adequate for determining carcinogenic potential in mice. There was a decrease in the survival of mice of both sexes at the highest dose level (2000 ppm), but the numbers of animals surviving to termination in all groups of both sexes are adequate for the assessment of carcinogenic potential. Body weight was significantly decreased (≈ 85%/73 % of control value) in both sexes at the 2000 ppm dose level, as was body-weight gain [significantly decreased at the 1000 (82 d/63 9 % of control value) and 2000 (64 d/63 9 % of control value) ppm dose levels in both sexes during the 0-13 week interval]. Food consumption was decreased at the highest dose level in both sexes also.
E. Additional Toxicology Data on Molinate:

1. Metabolism

Doses of unlabeled molinate were absorbed by the rat and extensively metabolized after both oral and i.v. administration (MRID# 41781801-41781804). The main route of elimination after 14 consecutive doses of 10 mg/kg was via the urine (79% %, 83% %). Ninety percent was excreted in 24 hours. Only a small percentage was excreted via the feces (3-10%) and expired air (1-1.5%). The highest tissue levels were found in the blood cells. At 96 hours post dose, 3.5% of the dose remained in the rat. A similar pattern was observed following single doses of either 10 or 100 mg/kg radiolabeled molinate; i.e., 70-72% was excreted via the urine, 8-11% via 5/5% via the feces, % expelled air, and blood showed the highest levels. Approximately 85% of the dose was recovered. A single i.v. dose of 1 mg/kg displayed a similar pattern of elimination.

Metabolism of molinate involves S-oxidation to form the intermediate molinate sulfoxide, which is either hydrolyzed to hexamethylenemine or conjugated with glutathione, ultimately forming mercapturic acid. Another significant route of metabolism involves ring hydroxylation at the 3 and 4 positions, followed by glucuronide conjugation. Ten of the 22 metabolites, which accounted for 74-86% of the urinary radioactivity, were isolated in the urine and identified:

- 4-hydroxyhexamethylenimine
- hexamethylenemine
- 3-hydroxyhexamethylenimine
- 4-keto-hexamethylenimine
- 3-hydroxy R-4572 glucuronic acid
- 4-hydroxy R-4572 glucuronic acid
- hydroxy R-4572 mercapturic acid

R-4572 mercapturic acid
3-hydroxy R-4572
4-hydroxy R-4572
R-4572

2. Mutagenicity

Molinate has been tested in several mutagenicity studies considered acceptable by the Agency. Molinate was negative for (1) mutagenic activity, with and without metabolic activation in Salmonella typhimurium, strains TA1535, TA1537, TA1538, TA98, and TA100 (MRID# 40918301) and (2) clastogenic activity in cultured human lymphocytes with and without metabolic activation (MRID# 40946701). In the mouse lymphoma assay with metabolic activation by both rat and mouse S9 activated systems, molinate was weakly mutagenic. A negative mouse micronucleus assay (MRID# 00163789) has been submitted, but not reviewed to date. Also, an aberration and sister chromatid exchange study with mouse lymphoma cells (MRID# 00163791) indicated suggestive, but not
reproducible increases with activation (also submitted but not reviewed to date).

Because of the indicated activity for three endpoints in the mouse lymphoma assays with activation (MRID 00163790), the observed germ cell interaction of molinate (see developmental and reproductive toxicity section), and the positive response in a published mouse bone marrow micronucleus test (Mutation Research 242:279-283, 1990), a dominant lethal study in the rat is required.

Additionally, a UDS assay (MRID 41052701) submitted is unacceptable, and the "Other Genotoxic Effects" category has not been satisfied. It is suggested that a sister chromatid exchange assay in germ cells or a UDS assay in germ cells be performed to fulfill this category, in light of the fact that the gonads are target organs of molinate.

3. Developmental and Reproductive Toxicity

In a rat developmental toxicity (1990) study (MRID 414734-01), maternal reproductive parameters were not affected by treatment of pregnant rats with molinate at dose levels of 2.2, 35, 140 mg/kg administered on days 6-16 of gestation. The NOEL for maternal toxicity was 35 mg/kg; the LOEL was 140 mg/kg, based on decreased body weight, body-weight gain, food consumption, increased salivation, and RBC cholinesterase inhibition. The NOEL for developmental toxicity was set at 2.2 mg/kg, the LOEL at 35 mg/kg, based on increased post-implantation loss, lower fetal body weight, and an increased incidence of runts and external/soft tissue/skeletal variants.

In a rabbit developmental toxicity (1985) study (MRID 140210-15), dose levels of molinate of 2, 20, and 200 mg/kg were administered on days 7 through 19 of gestation to sperm-positive female rabbits. There was an increase in the number of does that aborted and a decrease in the % of does with live fetuses at the 200 mg/kg dose level. The NOEL for maternal toxicity was set at 20 mg/kg, the LOEL at 200 mg/kg, based on decreased body weight, increased relative and absolute liver weight, and increased abortions. The NOEL for developmental toxicity was set at 20 mg/kg, the LOEL at 200 mg/kg, based on a decrease in the % of pregnant does with live fetuses at term.

In a 2-generation reproduction (1989) study in rats (MRID 413334-02), molinate was administered to females at dose levels of 6, 50, and 450 ppm prior to mating with untreated males, and through gestation, lactation, and weaning of their offspring for two generations. The systemic (maternal toxicity) NOEL was set at 6 ppm (0.3 mg/kg), the LOEL at 50 ppm (2.5 mg/kg), based on decreased body weight/body-weight gain, food consumption, and
increased adrenal weight. The reproductive toxicity NOEL was set at 6 ppm, the LOEL at 50 ppm, based on reduced fecundity and increased incidence of ovarian histopathological findings (vacuolation/hypertrophy).

Additionally, numerous studies have been performed in rats in which only the male was dosed with molinate. These studies were designed to address the antifertility effects of molinate. Untreated females were mated to treated males (various dose levels and duration of treatment), and some or all of the following parameters were monitored: # corpora lutea, # implants, # viable fetuses, # resorptions, T3, T4, TSH, LH, FSH, testosterone concentration, sperm viability/motility/concentration, adrenal, testes/epididymides weight and histology. Findings include: reductions in the number of implants/litter and viable fetuses/litter, decreases in implantation index, # pregnancies, sperm viability and motility, increases in preimplantation loss, resorptions/litter, sperm abnormalities.

The HED Peer Review Committee for Developmental and Reproductive Toxicity met on December 12, 1991 to discuss and evaluate the weight-of-the-evidence on molinate, with particular reference to its potential for reproductive and developmental toxicity. The Committee concluded that molinate causes effects on male reproduction in dogs, mice, and rats. The lowest NOEL is 0.2 mg/kg/day, found in the rat, based on effects on sperm measures and fertility. Female reproductive toxicity was observed in the rat, and a NOEL of 0.1 mg/kg/day was established on the histological changes observed in the ovary at higher dose levels. Because there are no data available under the conditions in which both male and female rats are dosed concurrently, further study of the reproductive toxicity of molinate was recommended.

4. Structure-Activity Correlations

Molinate is a thiocarbamate herbicide and structurally related to Triallate, Butylate, EPTC, Pebulate, Ethiolate, Thiobencarb, Vernolate, and Cycloate. These structures are shown in Figure 1.

Triallate was associated with an increased incidence of hepatocellular carcinomas in male mice fed dose levels up to 250 ppm for 2 years, with a positive trend in both sexes. The increase in the high-dose females was of borderline significance (at a less than adequate dose). There was an increase in kidney tubular cell adenomas in male rats fed dose levels up to 250 ppm for two years at all dose levels compared to the concurrent control, but statistical significance was attained only at the high-dose (250 ppm) level. In females, one rat (mid dose, 50 ppm) displayed one adenoma and one carcinoma. Both sexes showed an incidence greater than the historical control. The Peer Review Committee classified Triallate as Group C - possible human carcinogen and recommended that for the purpose of risk
characterization, a low-dose extrapolation model applied to the experimental animal tumor data should be used for quantification of human risk (Q*). Triallate was positive in *Salmonella*, *Saccharomyces cerevisiae*, mouse lymphoma cells, and sister chromatid exchanges in CHO cells; negative in another mouse lymphoma assay, an old dominant lethal study, and mutations in CHO cells. It should be noted, however, that Triallate may not be a good analog for Molinate. Triallate differs from Molinate in containing a 5-chloroallyl moiety which can generate, upon metabolic activation, highly mutagenic chlorinated acrolein. Triallate is a much more potent mutagen than molinate.

Butylate has been classified Category E, not a carcinogen at adequate dose levels. Butylate was negative with/without metabolic activation in the Ames assay, DNA repair test (*Sacc. cerevisiae*), and mouse lymphoma (forward mutation); induced dose-related sister chromatid exchange with activation only in mouse lymphoma assay (cytogenetic), but no chromosomal aberration with/without activation; negative for ability to transform BALB/c 3T3 cells.

EPTC (*Eptam*) showed no evidence of carcinogenicity in either the rat or mouse when tested at adequate dose levels, but it was not classified by HED. *Eptam* was negative in the Ames (*Salmonella*) assay with/without metabolic activation, in the micronucleus test, sex link recessive (*Drosophila*), and chromosomal aberration assay; induced a positive mutagenic effect with S9 in the mouse lymphoma assay and was positive for gene mutation (TK locus) with metabolic activation (negative without).

Pebulate was negative for carcinogenicity in the rat at adequate dose levels, but it was not classified by HED; no mouse study was located. No chronic toxicity/carcinogenicity studies were located for Ethiolate. No mutagenicity studies were located for Ethiolate or Pebulate.

Thiobencarb was negative for carcinogenicity in both the mouse and rat studies, although there was a dose-related increase in the number of testicular interstitial cell adenomas, which was statistically significant at the highest dose tested (500 ppm) compared to the concurrent control value [incidence: control 76.6%, low-dose 85.0%, mid-dose 88.3%, and high-dose 90.0%]. The review of the study indicated that these percentages were within historical control data. It was not classified by HED. Thiobencarb was not mutagenic in the Ames assay or in human lymphocytes with/without activation; but was mutagenic in the mouse micronucleus assay.

Vernolate was not carcinogenic in mice, but was not classified by HED. The systemic NOEL >100 mg/kg (HDT). No rat study on Vernolate was located. Vernolate induced a positive response in
sister chromatid exchange with/without activation in the only acceptable study located.

Cycloate (Ro-Neet) was negative in both the rat and mouse carcinogenicity studies, but was not classified by HED. Ro-Neet was positive for forward mutation under activated conditions only (at toxic level), for chromosomal aberrations and sister chromatid exchange induction under activated conditions; previously reported negative for chromosomal aberrations; negative in mouse micronucleus test.
Figure 1

Structural Analogs of Molinate

Molinate
(Ordram)

EPTC
(Eptam)

Butylate
(Sutan)

Ethiolate

Vernolate
(Vernam)

Pebulate
(Tillam)

Thiobencarb
(Saturn)

Triallate

Rico-Mest
(Cycloate)
5. Acute, Subchronic, and Chronic Toxicity Studies

The acute oral LD₅₀ for Ordram® Technical in rats is approximately 550 mg/kg; Tox. Cat. III, (caution). In a 1965 13-week feeding (8, 16, & 32 mg/kg) study in rats, ovarian vacuolation was observed at 16 mg/kg (LEL); the NOEL was set at 8 mg/kg. Increased thyroid weight was observed in a 13-week dog study (doses of 450, 900, & 1800 ppm); the NOEL was 900 ppm. No significant systemic toxicity was observed in a 3-week dermal study of rats using dose levels of 10, 25, & 50 mg/kg.

In a 3-month inhalation study in rats at dose levels of 2, 10, & 50 mg/m³, testicular degeneration and abnormal spermatozoa were observed at 2 mg/m³; no NOEL was determined. Animals exposed to 50 mg/m³ exhibited decreased weight gain, mucus discharge, rapid breathing, decreased brain cholinesterase, decreased reticulocytes, increased adrenal weight, and decreased pituitary weight.

A one-year dog study was performed at concentrations of 0, 1, 10, 50, & 100 mg/kg/day (gelatin capsules) (MRID# 417811-01). Due to toxicity, the 100 mg/kg group animals were dosed for only 14 weeks. Body weight and body-weight gain were decreased at the two highest dose levels in both sexes, although the females did not attain statistical significance during most of the study. However, the magnitude of the decrease was >10% below the control value at all dose levels in females and at the two highest dose levels in males from week 8 on.

There was a dose-related increase in relative (to body weight) kidney weight at all dose levels in females dosed for the entire study, and brain weight was decreased in females at 10 mg/kg and in both sexes at 50 mg/kg (dose-related). Adrenal weight was increased in both sexes at the 50 mg/kg level (relative to brain weight in both sexes; relative to body weight in females), and liver weight (relative to body weight) was increased in both sexes at the 50 mg/kg dose level and at the 10 mg/kg dose level in females; liver weight relative to brain weight was increased only in the 50 mg/kg males compared to the control values.

Decreases in sperm ejaculate, reduction in the percentage of motile sperm, and suggestive testicular atrophy were observed. At three months, the dogs were anemic, and dogs at the 50 and 100 mg/kg dose level lost the ability to bark or had an attenuated bark. Signs of neurotoxicity included ataxia, splayed hind limbs, vacuolation of the medulla, demyelination of the pons and spinal cord, and tremors. Additionally, eosinophilic bodies were observed in the nervous system. The NOEL for effects other than neurotoxicity was set at 10 mg/kg, the LEL at 50 mg/kg, based on decreased body-weight gain, anemia, and decreased ejaculate volume/% of motile sperm.
Because histological lesions in the sciatic nerve/spinal cord occurred at all dose levels in this study and histopathological lesions in the nerve/muscle tissue were displayed at all dose levels in the rat study (discussed above), a definitive NOEL for these effects is not available. During the FIFRA review, it was determined that an acute and subchronic neurotoxicity screen/developmental neurotoxicity screen would be required on molinate.
F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on molinate in a weight-of-the-evidence determination of carcinogenic potential:

1. Administration of molinate in a 2-year chronic toxicity/carcinogenicity study was associated with a statistically significant increase in combined kidney adenomas and/or carcinomas by pairwise comparison at the high dose (300 ppm) in male Crl:CD®(SD)BR rats. There was also a statistically significant positive trend for kidney carcinomas and combined adenomas and/or carcinomas. There was no increase in tumors in female rats. The increased incidence of kidney tumors in the male rat at the high-dose level exceeded the available historical control data (9 studies) for both adenomas and carcinomas. These are considered to be rare tumors.

2. There was a statistically significant positive trend for mesotheliomas in the testes of male rats. Testicular interstitial cell tumor incidence exceeded the historical control incidences, and the increase was observed at all dose levels. The PRC determined that the evidence for interstitial testicular cell tumors is equivocal, since there was no increase in trend or pair-wise comparisons. The PRC concluded that the doses used in the rat study were adequate for assessing the carcinogenic potential of molinate. Supportive evidence that the testes is a target organ for this chemical is the finding of adverse reproductive effects. The incompletely reported Japanese study indicates that the testes is a site for tumors caused by molinate, and also lends support to the presumption that the tumors are compound-related.

3. There was a statistically significant positive trend for combined liver adenomas and carcinomas in the male rat, but the PRC determined that the incidences were within historical controls, and were not due to administration of molinate.

4. In mice, there was no compound-related increase in tumors observed in either sex at dose levels of molinate up to 2000 ppm, which were considered adequate, based on increased mortality, decreased body-weight gain, and increased incidence of histological lesions in the testes and ovaries.
5. Molinate was negative in a Salmonella assay and for aberrations in cultured human lymphocytes. However, suggestive increases were found for mutations, aberrations and SCE in mouse lymphoma cells. While a submitted mouse micronucleus assay was negative, a published report at similar dose levels demonstrated a strong response. All this suggests molinate has some clastogenic potential. In conjunction with the reproductive effects showing germ line effects, a dominant lethal test is required.

6. Molinate is structurally related to (1) Triallate, which has been classified as Group C (possible human carcinogen) with a Q; (2) Butylate, which has been classified as Category E, not a carcinogen; (3) Thiobencarb, which was negative for carcinogenicity in both the mouse and rat studies; (4) Cycloate, which was negative in both the mouse and rat studies. For the other structurally-related compounds the database is incomplete, but the available studies appear to be negative. No chronic toxicity/carcinogenicity studies were located for Ethiolate and only a rat study was located for Pebulate and a mouse study for Vernolate.
G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity.

The Peer Review Committee agreed that the classification for molinate should be Group C - possible human carcinogen and recommended that for the purpose of risk characterization, a low dose extrapolation model applied to the experimental animal tumor data should be used for quantification of human risk ($q_*$).

This decision was based on a statistically significant increase in combined adenomas and carcinomas in the male rat kidney. This is considered to be a rare tumor type. There was equivocal evidence that molinate induced an increase in testicular tumors. No increases were noted in liver tumors. No increases in tumors were found in female rats. This study was conducted using adequate doses for the determination of carcinogenic activity.

No increases in tumors were found in mice of either sex. Molinate is structurally related to several compounds. Only triallate was positive for carcinogenicity, but its mechanism of action may be different from molinate. The evidence for mutagenicity is weak. The PRC is not aware of any human evidence on the carcinogenicity of molinate.

The PRC decided that the $q_*$ should be based on the incidence of combined tumors in the male rat kidney.