Select Comments to the DART-IC on Chlorsulfuron Relevant to Proposition 65 Delisting

May 21, 2014

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Chlorsulfuron Overview

- Developmental conclusions
  - Studies in rabbits
  - Study in rats

- Reproduction conclusions
  - Reproduction studies in rats
Developmental toxicity conclusions

- The original finding of increased resorptions in rabbits was not reproduced in a guideline study using a more robust design & higher dose levels.

- Effects observed in the replacement studies have been clarified to be the result of:
  - Increases in offspring number influencing the weight of fetal rabbits
  - Maternal toxicity effecting fetal weight in the case of the rats
Developmental toxicity – Alvarez (1991a) rabbit study

- Chlorsulfuron on GD 7-19, 20 does/dose, 0.5% methyl cellulose:
  - 0, 25, 75, 200 or 400 mg/kg/day (original study)
  - 0, 400 or 1000 mg/kg (supplement study)
- No increase in resorptions at any dose; not even at 1000 mg/kg
- Slight decreases in fetal body weight at 400 mg/kg, not considered biologically significant, since within the historical control range
- U.S. EPA noted the decrease in weight might be attributed to ↑ offspring number*, so we put this to the test in a supplement to Alvarez**
  - Analysis of Covariance tested correlation between fetal body weight & either dose or pup number
  - Fetal body weight
    - did not correlate with dose
    - did correlate with number of pups

**Munley 2012, Supplement 3 to Alvarez
Teratology Study in Rabbits (Hoberman et al., 1980)

- Chlorsulfuron in corn oil: 0, 10, 25 or 75 mg/kg/day on GD 6-19, sacrificed GD 29
- Resorption rate at the top dose (75 mg/kg) was higher than concurrent control & reported as test substance-related
- U.S. EPA eventually required a new study as the 1980 study was guideline deficient
  - Low # of animals - guideline calls for ~20 animals/group with implantation sites at necropsy
  - Hoberman had 16/17 does/dose with only 12/13 to evaluate in high dose and control
- When number of animals is low, historical control data is more critical, hence a supplement was made....
Munley 2014: 
Supplement 1 Revision 2 of (Hoberman et al., 1980)

- Compared results to historical control data, MARTA* Data (performing lab contributed data)
- Small litters may have lower hormone levels, which could make it difficult for does to sustain pregnancy, leading to resorptions**
- Assessed data with & without totally resorbed litters
  - MARTA data likely a mixture of with & without 100% resorptions
  - Next slide shows the impact of 1 doe at 10 mg/kg & 75 mg/kg each with 100% resorptions

*Middle Atlantic Reproduction & Teratology Association, Lang, PL, editor (1993). HRP Inc. Performing lab is listed as a participant
<table>
<thead>
<tr>
<th>Dose: mg/kg/day</th>
<th>0</th>
<th>10</th>
<th>25</th>
<th>75</th>
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<tbody>
<tr>
<td><strong>All pregnant rabbits included</strong></td>
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<tr>
<td>Rabbits / # pregnant</td>
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<tr>
<td>N</td>
<td>16 / 13</td>
<td>16 / 14</td>
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<td>17 / 16</td>
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<tr>
<td>Rabbits evaluated</td>
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<tr>
<td>N</td>
<td>13</td>
<td>14</td>
<td>16died GD 18</td>
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<tr>
<td>Mean # resorptions/litter (SD)</td>
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<tr>
<td>0.7 (1.2)</td>
<td>1.4 (1.7)</td>
<td>0.7 (1.0)</td>
<td>2.1 (2.0)</td>
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<tr>
<td><strong>Mean % resorptions/litter (SD)</strong></td>
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<tr>
<td>11.6 (23)</td>
<td><strong>23.9 (32)</strong></td>
<td>8.5(12)</td>
<td><em><em>33.7</em>(28)</em>*</td>
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<tr>
<td><strong>Pregnant rabbits with 100% resorptions excluded</strong></td>
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<td>Rabbits / # pregnant</td>
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<td>N</td>
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<td>1died GD 18</td>
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<td>Rabbits evaluated</td>
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<td>N</td>
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<td>0.7 (1.2)</td>
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<tr>
<td>11.6 (23)</td>
<td><strong>18.0 (24)</strong></td>
<td>8.5 (12)</td>
<td><strong>28.2 (21)</strong></td>
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<tr>
<td><strong>MARTA Historical Control Data</strong></td>
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<tr>
<td>Mean # resorptions</td>
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<td></td>
</tr>
<tr>
<td>Mean of means: 0.6</td>
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<tr>
<td>Range: 0 - 3.2</td>
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<tr>
<td>Mean % resorptions/litter**</td>
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<tr>
<td>Mean of means: 8.2</td>
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<tr>
<td>Range: 0 – 47.3^</td>
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</tbody>
</table>

*Statistically significant
@ One dead GD 12, one euthanized in extremis, one with scars-previous pregnancy
^ Mean # resorptions in a group / # of litters
**Group mean of [(resorptions per litter / implantations per litter) x 100]
^^Range high values: 29.2 for D29 studies, 47.3 for D28 studies. Hoberman covered both ranges
Hoberman et al., 1980: Maternal effects

Maternal toxicity not clearly described by the author & the dosing regimen allowed for weight recovery; however, data during dosing is available in the report

- One death at 25 mg/kg
- Two deaths/dying at 75 mg/kg
- Range finder: 2/4 died at 100 mg/kg & 4/4 ≥300 mg/kg*

- Gross pathology changes increased with dose, pale liver & kidney & nutmeg liver most often noted (suggesting hepatic congestion)
- Rabbits were large suggesting they may have been old; hence, age may have impacted the data

Food consumption & weight changes during dosing re-analyzed in detail

**Hoberman et al., 1980: Maternal effects**

- Frequency of does with 0-20 g/day food intakes over GD 7-20

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th># Animals</th>
<th>Number of low food intakes (≤ 20 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td>25</td>
<td>17</td>
<td>29</td>
</tr>
<tr>
<td>75</td>
<td>17</td>
<td>62</td>
</tr>
</tbody>
</table>

- Similar to finding in the literature which report a correlation between ↑ resorptions & ↓ food intake*

- With chlorsulfuron body weight ↓ during dosing, esp. at 75 mg/kg:
  - 8 does ↓ ≥300 g during dosing; of those, 4 dropped >400 g
  - These marked weight decreases are not easily noted due to the study design, as weights recovered after dosing

- Decreased food intake & body weight, along with the deaths demonstrate that 75 mg/kg was maternally toxic

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Developmental Toxicity Studies in Rabbits: Summary of resorptions

- Conclusions based on the top dose in both studies are difficult due to low animal numbers - 1980 study design & both studies mortality
- Mean % resorptions/litter was within or slightly over the historical control range; always within range for mean # resorptions
- No dose response for resorptions in 1980 study
- Resorptions at 75 mg/kg were
  - Influenced, in part, by a few does with low numbers of implants*
  - Either spurious, or if test substance-related, occurred in the presence of significant maternal toxicity
- No increase in resorptions in guideline 1991 study, with a robust number of animals & a broad dose range
- ↑ resorptions by chlorsulfuron are no longer considered relevant in U.S. EPA’s documents

*1 doe at 75 mg/kg had 1 implant which totally resorbed, another had 2 implants & 1 resorbed
Teratogenicity study in rats, Alvarez, 1991b

- Not used as the basis for TRI Listing by U.S. EPA
- Recently reviewed by the U.S. EPA – not of concern
- U.S. EPA’s definition of maternal toxicity in its 1991 Guidelines for Developmental Toxicity Risk Assessment:

  “Agents that produce developmental toxicity at a dose that is not toxic to the maternal animal are esp. of concern…However, the more common situation is when adverse developmental effects are produced only at doses that cause *minimal maternal toxicity* [marginal but significantly reduced body weight, reduced weight gain, or specific organ toxicity, and at the most no more than 10% mortality]; in these cases, the developmental effects are still considered to represent developmental toxicity and should not be discounted as being secondary to maternal toxicity.”

*Emphasis added*
Teratogenicity study in rats (Alvarez, 1991b)

- 25 rats/dose mated with males, gavaged with chlorsulfuron in 0.5% MC at 0, 55, 165, 500 or 1500 mg/kg on GD 7-16, sacrificed GD 22

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>0</th>
<th>55</th>
<th>165</th>
<th>500</th>
<th>1500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean fetal weight (g)</td>
<td>5.44</td>
<td>5.58</td>
<td>5.48</td>
<td>5.34</td>
<td>4.91*</td>
</tr>
</tbody>
</table>

- Mean fetal weight ↓ 10% at 1500 mg/kg
- Maternal weight recovers somewhat, since dosing stops on GD 16, but during dosing – next slide
Teratogenicity study in rats, (Alvarez, 1991b) cont.

- Maternal effects
  - **500 mg/kg:** vaginal discharge, ↓ food intake, ↓ weight gain
  - **1500 mg/kg:**
    - 2 treatment-related deaths,
    - Findings as with 500 mg/kg, but more pronounced:
      - vaginal discharge,
      - stained perineum,
      - swollen limbs & face,
      - perinasal staining,
      - ↓ food intake 18-33% over GD 7-17,
      - ↓ adjusted final body weight 4%,
      - weight gain ↓ 50% over GD 7-17,
      - ↓ adjusted weight gain significantly different 30% ↓ over GD 7-22
Teratogenicity study in rats, (Alvarez, 1991b) cont.

1998 U.S. EPA Guideline *Prenatal developmental Toxicity Study*, Dose levels & selection:

“the highest dose should be chosen with the aim to induce some developmental and/or maternal toxicity *but not death or severe suffering*. …maternal mortality…not be more than ~10% …the highest dose tested need not exceed 1000 mg/kg/day…higher levels of death may invalidate the study”

- Based on today’s standards, the findings at 1500 mg/kg are considered overly toxic, not minimally toxic
- The decrease in fetal body weight can be clearly attributed to overt maternal toxicity
Female & male reproductive toxicity conclusions:

- 1981 3-generation study retrospective analyses demonstrated:
  - Fertility index was within the historical control range
  - Fertility index was not a statistically significant finding

- 2005 2-generation study:
  - Dosing 3-fold higher than 1981 study
  - No test substance-related changes in reproductive parameters
Reproductive toxicity


- U.S. EPA Reproductive Assessment Guidance of 1993 recommends statistical tests which were not routinely used in 1981; those tests suggests that the finding is spurious
Reproductive toxicity – new study

- DuPont conducted a guideline-compliant reproduction study in rats (Mylchreest 2005)
  - Highest dose was 7500 ppm *versus* 2500 in the previous study
  - Two generations according to guideline - no current guidelines use 3 generations
  - Only adverse effects: decreases in parental body weight, weight gain & food efficiency
  - No test substance-related effect on fertility or any reproductive parameters seen at 7500 ppm
  - Included more reproductive parameters such as sperm motility
### Reproductive toxicity – 2005 study

**Fertility Index (# pregnant/copulated)**

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>0</th>
<th>100</th>
<th>500</th>
<th>2500</th>
<th>7500</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P1 generation</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Fertility index</td>
<td>88.5</td>
<td>89.3</td>
<td>93.3</td>
<td>96.7</td>
<td>93.3</td>
</tr>
<tr>
<td></td>
<td>(23/26)</td>
<td>(25/28)</td>
<td>(28/30)</td>
<td>(29/30)</td>
<td>(28/30)</td>
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<tr>
<td><strong>F1 generation</strong></td>
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<tr>
<td>Fertility index</td>
<td><em>81.5</em></td>
<td>88.5</td>
<td>92.6</td>
<td>96.6</td>
<td>78.6</td>
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<tr>
<td></td>
<td>(22/27)</td>
<td>(23/26)</td>
<td>(25/27)</td>
<td>(28/29)</td>
<td>(22/28)</td>
</tr>
</tbody>
</table>

*From tables 53 and 54*
Reproductive toxicity – Supplement to 1981 Study

- Revised the study:
  - Put results in context of historical control data & the 2005 study
  - Perform statistical tests as recommended in EPA’s Standard Evaluation Procedures for Reproductive Toxicity Studies

- Historical control data show the fertility index of 79% falls in the historical range reported by the lab (60 to 100%) & is comparable to control results from the 2005 study (F1 control 81.5%)

- The data were not statistically significant using U.S. EPA recommended tests

- The original study director did not have the advantage of historical control data & the statistical tools that would be recommended later
Reproductive toxicity – Supplement to 1981 Study, cont.

- Examination of the two matings producing the 3rd generations showed that all females were fertile in at least one of the two pairings performed in the 3rd generation.
- Three males were unsuccessful in mating in both F3 generations; similar findings were seen in control males in a 1983 study at the same lab (4/20 infertile).
- In line with in-breeding problems reported to Charles River (main breeder of SD rats).
- Charles River then developed a practice of proving the fertility of males before using in fertility tests.
- Led to the re-derivation of this rat strain.
Thank You