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

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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

*U.S. EPA DER (2002) p. 110
**Munley 2012, Supplement 3 to Alvarez

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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….

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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

**Fuessner, EL *et al*. (1992). A decade of rabbit fertility data: study of historical control animals, *Teratology*, 46:349-365

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Munley 2014, Supplement 1 Revision 2 of Hoberman et al., 1980

Dose: mg/kg/day		0	10	25	75
All pregnant rabbits included					
Rabbits / # pregnant	N	16 / 13	16 / 14	17 / 17	17 / 16
Rabbits evaluated	N	13	14	16 died GD 18	13@
Mean # resorptions/litter^ (SD)		0.7 (1.2)	1.4 (1.7)	0.7 (1.0)	2.1 (2.0)
Mean % resorptions/litter** (SD)		11.6 (23)	**23.9 (32)**	8.5(12)	**33.7*(28)**
Pregnant rabbits with 100% resorptions excluded					
Rabbits / # pregnant	N	16 / 13	16 / 14	17 / 17	17 / 16
# 100% resorptions	N	0	1	1 died GD 18	1
Rabbits evaluated	N	13	13	16	12@
Mean # resorptions/litter^ (SD)		0.7 (1.2)	1.2 (1.6)	0.7 (1.0)	2.2 (2.0)
Mean % resorptions/litter** (SD)		11.6 (23)	**18.0 (24)**	8.5 (12)	**28.2 (21)**
MARTA Historical Control Data					
Mean # resorptions^		Mean of means: 0.6		Range: 0 - 3.2	
Mean % resorptions/litter**		Mean of means: 8.2		Range: 0 – **47.3**^^	

* 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

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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, A. (1980). Pilot Teratogenicity Study in Rabbits, Haskell #12,700, Final Report. Hazelton Laboratories America, Inc. Project No. 201-535.

Hoberman et al., 1980: Maternal effects

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

Dose (mg/kg/day)	# Animals	Number of low food intakes (≤ 20 g)
0	16	10
10	16	25
25	17	29
75	17	62

- 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**

*e.g., Matsuoka, T., *et al*. (2006) *J Toxicological Sciences*, 31(2): 169-175 and Clark, RL *et al*. (1986) *Fund Appl Toxicol*. 7:272-286

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

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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

Dose (mg/kg)	**0**	**55**	**165**	**500**	**1500**
Mean fetal weight (g)	5.44	5.58	5.48	5.34	4.91*

- Mean fetal weight ↓ 10% at 1500 mg/kg
- Maternal weight recovers somewhat, since dosing stops on GD 16, but during dosing – *next slide*

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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

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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

- 1981 reproduction study in rats reported ↓ fertility (Wood, 1981) leading to U.S. EPA's TRI Listing

- 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)

Concentration (ppm)	**0**	**100**	**500**	**2500**	**7500**
P1 generation Fertility index	88.5 (23/26)	89.3 (25/28)	93.3 (28/30)	96.7 (29/30)	93.3 (28/30)
F1 generation Fertility index	**81.5** (22/27)	88.5 (23/26)	92.6 (25/27)	96.6 (28/29)	78.6 (22/28)

*From tables 53 and 54

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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

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