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Tox-3/55

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

003155

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: Richard Mountfort
Product Manager No. 23
Registration Division (TS-767C)

THRU: Christine F. Chaisson, Ph.D. Christine F. Chaisson 8/16/83
Acting Deputy Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

SUBJECT: Teratology (6-a-2) Data on Acrolein, Registration Action No. 10707-9.

Action Requested:

Magna Corporation has submitted two teratology studies (rat and mouse) for review under FIFRA, Section 6(a)(2). Magna reports that "... In summary, acrolein appears to contribute to the increase in a number of visceral and skeletal anomalies in the CD-1 mouse. In the Sprague-Dawley rat, acrolein produced several minor anomalies, as well as reduced fetal weight. In both studies, it should be noted that the significant findings in the fetus accured at levels which were toxic to the maternal animal."

Review of Teratology Studies:

The following precis of these reports presented here are meant to serve as a basis for understanding the conclusions reached and the recommendations made after a review of the 2 teratology studies. Detailed reviews of the individual studies are attached for a more in-depth analysis of the data.

Report 1:

King, M. and J.A. Salinas; "Teratology study of acrolein in mice" Bioassay Systems Corporation Project No. 12058, submitted by Magna Corporation, Houston, Texas, 1 September 1982.

In this teratology study, technical grade acrolein (>96% pure) was diluted with deionized water and administered by gastric intubation to mated, female CD-1 mice (32/group) at dosages of 4.0, 6.3, or 10.0 mg/kg on gestational days 7-17. Control dams received deionized water only. At terminal

1 of 42

003155

sacrifice, the body weight gains of dams in all 3 acrolein-treated groups were significantly smaller than controls. Based on these data, a maternal systemic NOEL for acrolein in pregnant mice was not established (<4 mg/kg).

No ill effects were observed in fetal viability, fetal weights, or male:female sex ratios. The incidences of fetuses exhibiting at least 1 anomaly were higher in all 3 treated groups than in the control group. The preponderance of anomalies was due to minor fetal aberrations (dilated renal pelves; delayed ossifications of sternebrae, metacarpals or metatarsals; subcutaneous edema; 14th rib). The overall incidence of major malformations did not differ among all 4 groups; however, 4 cases of cleft palate occurred in the acrolein-treated groups (2 each in the mid- and high-dose groups). As discussed below, the paucity of critical, teratologic data precludes drawing conclusions concerning the teratogenicity of acrolein in mice from this study at this time. It remains unclear if cleft palate is a possible teratogenic effect of acrolein. Historical data on cleft palate in this species would aid in the evaluation of these test results. However, acrolein was demonstrated to be embryotoxic at doses as low as 4 mg/kg (Lowest Dose Tested) based upon the overall increased incidence of both major and minor anomalies among all acrolein-treated groups. Therefore, a fetotoxic NOEL was not established in this study (NOEL < 4 mg/kg).

CORE CLASSIFICATION: Supplementary. This study exhibited numerous short-comings:

1) Maternal toxicity was observed at all doses, even the lowest; therefore, a NOEL for maternal toxicity was not established.

2) Fetotoxicity (primarily generalized delayed ossification of fetuses) was observed at lowest dose tested; therefore, the fetotoxicity NOEL was not established.

3) The animals were treated beyond the stage of organogenesis; dosing should have been stopped at day 16.

4) Too few pregnant animals were analyzed in this study (range 12-20 litters/group).

5) The complete description of the chemical tested was not given; 4% unspecified.

6) Several important types of data were not reported:

- maternal food consumption
- numbers of corpora lutea
- individual fetal body weights

7) Only one-half of the fetuses were examined for cleft palate. The registrant should be requested to examine all skeletal preparations for the presence or absence of clefts of the hard palate.

8) The animals were killed too early; they should have been sacrificed on day 19 vice day 18.

9) Sex was determined in only one-half of the fetuses.

10) Maternal body weights were measured only on the first day of dosing and at terminal sacrifice.

Data Requested:

This study cannot be upgraded to CORE minimum due to primarily 1 and 2 above. Therefore, no additional data from this study is required. However, several issues should be addressed in any future teratology study on acrolein:

1) All the deficiencies (1-10) noted above should be taken into consideration and resolved.

2) Since the cleft palate observed may be due to treatment with acrolein, all skeletal preparations should be examined for the presence or absence of clefts of the hard palate (see 7 above).

3) The submissions of historical control data from the same laboratory would aid in the evaluation of any test results.

4) [redacted]

5) The reference given for skeletal staining (Inouye, 1976) describes a method for combining staining of cartilage and bone. Did the authors in fact perform dual staining? If so, many of their putative aberrations should be more accurately scored. If they did not perform dual staining, what method did they use?

Report No. 2:

King, M. and T.C. Crowell: "Teratology study of acrolein in rats" Bioassay Systems Corporation Project No. 10258, submitted by Magna Corporation, Houston, Texas, 12 November 1982.

In this teratology study, technical grade acrolein (>96% pure) was diluted with deionized water and administered by gastric intubation to groups of 40 mated, female CD rats at dosages of 3.6, 6.0, or 10.0 mg/kg on gestational days 7-19. Control dams received deionized water only. At terminal sacrifice, the body weight gains of dams in the mid- and high-dose acrolein-treated groups were significantly smaller than controls. In addition, these 2 groups exhibited increased mortality. Based on these data, the maternal systemic LEL for acrolein in pregnant rats is 6.0 mg/kg; the NOEL is 3.6 mg/kg.

No ill effects were observed in fetal viability, or male:female sex ratios. The mean fetal weight of the high-dose groups was significantly smaller than controls. The incidences of fetuses exhibiting at least 1 anomaly were high in the control and all 3 treated groups. The preponderance of anomalies was do to minor fetal aberrations, primarily delayed ossifications. The overall incidence of major malformations (runts) was significantly greater than controls ($p \leq 0.01$) in the high-dose group. The most common malformation was the presence of fetal runts. From these data, the teratogenic and fetotoxic LEL is estimated to be 10.0 mg/kg; the NOEL is 6.0 mg/kg. Therefore, the teratogenic and fetotoxic effects observed in rats were at doses higher than the maternal toxic NOEL.

CORE CLASSIFICATION: Core Minimum. This study exhibited numerous short-comings:

1) Several important types of data were not reported:

- maternal food consumption
- numbers of corpora lutea
- individual fetal body weights

003155

2) The chemical tested was not completely defined; 4% was unspecified.

3) The rats were treated beyond the stage of organogenesis; dosing should have been stopped at day 16.

4) The animals were killed too early; they should have been sacrificed on day 21 versus day 20.

5) Sex was determined for only one-half of the fetuses.

6) Maternal body weights were measured only on the first day of dosing and at terminal sacrifice.

Data Requested:

This study was classified as CORE MINIMUM; the data are adequate to establish 1) a maternal toxicity NOEL of 3.6 mg/kg; LEL = 6.0 mg/kg and 2) a teratogenic and fetotoxic NOEL of 6.0 mg/kg; LEL = 10 mg/kg. Therefore, no additional data are required on this study at this time. However, in any future teratology study on acrolein, several important issues should be addressed:

1) All deficiencies (1-6) noted above should be taken into consideration and resolved.

2) The submission of historical control data from the same laboratory would aid in the evaluation of any test results.

3) [redacted]

4) The reference given for skeletal staining (Inouye, 1976) describes a method for combining staining of cartilage and bone. Did the authors in fact perform dual staining? If so, many of their putative aberrations could be more accurately scored. If they did not perform dual staining, what method did they use?

003155

Conclusions:

1) Mice: Due to the deficiencies noted in the mouse study, no definitive conclusions may be reached concerning the possible teratogenic effect of acrolein in this species. However, there is some indication that cleft polate is a teratogenic effect of acrolein in mice and this issue should be addressed in any future teratology study on acrolein. It would aid in the inerpretation of the data if the registrant a) examined all skeletal preparations for the presence or absence of clefts of the hard palate and b) submitted historical control data on the incidence of cleft palate in this species in their laboratory. CORE SUPPLEMENTARY.

2) Rat: Acrolein was demonstrated to cause teratogenic and fetotoxic effects (NOEL = 6 mg/kg; LEL = 10 mg/kg) only at doses which exceeded the maternal toxic NOEL (3.6 mg/kg; LEL = 6 mg/kg). Core Minimum.

Chad B Sandusky 8/15/83

Chad B. Sandusky, Ph.D.
Pharmacologist
Toxicology Branch
Hazard Evaluation Division
(TS-769C)

attachments: 2

003155

* CONFIDENTIAL BUSINESS INFORMATION *

MRID: Not assigned

1. Chemical or Chemicals:

Acrolein

2. Type or Formulation:

Technical (96%)

3. Citation or Citations:

King, M. and T.C. Crowell "Teratology study of acrolein in rats" Bioassay Systems Corporation Project No. 10258, submitted by Magna Corporation, Houston, Texas, 12 November 1982

4. Reviewed by:

John M. DeSesso
Systems Scientist
The MITRE Corporation
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Signature: John M. DeSesso
Date: 4 March 83

5. Approved by:

Chad B. Sandusky
Pharmacologist
Toxicology Branch (TS-769)
Hazard Evaluation Division
Office of Pesticides and Toxic Substances
U.S. Environmental Protection Agency

Signature: Chad B. Sandusky
Date: 3/4/83

6. Discipline/Topic or Test Type:

This study has information pertinent to discipline toxicology, TOPIC TERATOGENICITY.

This study relates to the Proposed Guidelines data requirement 163.83-3.

* CONFIDENTIAL BUSINESS INFORMATION *

7. Conclusions:

In this teratology study, technical grade acrolein (>96% pure) was diluted with deionized water and administered by gastric intubation to groups of 40 mated, female CD rats at dosages of 3.6, 6.0, or 10.0 mg/kg on gestational days 7-19. Control dams received deionized water only. At terminal sacrifice, the body weight gains of dams in the mid- and high-dose acrolein-treated groups were significantly smaller than controls. In addition, these 2 groups exhibited increased mortality. Based on these data, the maternal systemic LEL for acrolein in pregnant rats is 6.0 mg/kg; the NOEL is 3.6 mg/kg.

No ill effects were observed in fetal viability, or male:female sex ratios. The mean fetal weight of the high-dose groups was significantly smaller than controls. The incidences of fetuses exhibiting at least 1 anomaly were high in the control and all 3 treated groups. The preponderance of anomalies was due to minor fetal aberrations, primarily delayed ossifications. The overall incidence of major malformations (runts) was significantly greater than controls ($p \leq 0.01$) in the high-dose group. The most common malformation was the presence of fetal runts. From these data, the teratogenic LEL is estimated to be 10.0 mg/kg; the NOEL is 6.0 mg/kg.

CORE CLASSIFICATION: Core Minimum. This study exhibited numerous short-comings:

- several important types of data were not reported
  - maternal food consumption
  - numbers of corpora lutea
  - individual fetal body weights
- the chemical tested was not completely defined; 4% was unspecified
- the rats were treated beyond the stage of organogenesis; dosing should have been stopped at day 16
- the animals were killed too early; they should have been sacrificed on day 21 versus day 20
- sex was determined for only one-half of the fetuses
- maternal body weights were measured only on the first day of dosing and at terminal sacrifice

8. Materials and Methods:

Technical grade Acrolein (CAS No. 000107028; purity 96%) was obtained from Magna Corporation, Santa Fe Springs, CA. The material was a colorless liquid which was stored in a sealed container under argon at room temperature. The complete chemical composition of the test material was not supplied by Magna Corporation.

A total of 160 timed-pregnant Sprague-Dawley CD rats were purchased from Charles River Breeding Laboratories, Kensington, New York. The animals arrived at the testing facilities of Bioassay Systems Corporation on gestational day 5; the day that mating was confirmed was designated day 1 of gestation. Neither details of the

mating procedures nor the means for confirmation of pregnancy were available. Upon arrival, the rats were 8-10 weeks old and weighed 169-230 g. They were identified by animal numbers which were marked by an ear punch method. Each rat was housed individually in polycarbonate cages containing hardwood chip bedding (Sani-chips) in a climate controlled animal facility. Ambient temperature was maintained at 21-23°C; the relative humidity was 46-61%; and the light/dark cycle allowed 12 hours of light per day. Air flow in the animal quarters varied from 12-16 complete changes of charcoal filtered fresh air each hour. Throughout the study, rats were allowed free access to Charles River Pelleted Rodent Diet and tap water.

Upon receipt on gestational day 5, the animals were quarantined for 2 days and observed daily for signs of disease. On gestational day 7, the rats were assigned to one of the 4 treatment groups (40 rats/group) by means of a random numbers program. The experimental group and dosage level were recorded on each animal's cage card.

The rats received daily doses of the test compound by gastric intubation on gestational days 7-19. Based upon each rat's body weight on gestational day 7, test dosages of 3.6, 6.0, or 10.0 mg/kg of acrolein were dissolved in sufficient deionized water to ensure that the volume of each dose was 5 ml/kg body weight. Formulations

of stock treatment solutions were stored in the dark under argon at room temperature for not more than 5 days. Fresh stock solutions were prepared 4 times during the study. Control animals received deionized water only.

During the period of testing, the rats were observed daily for clinical signs. Each rat was weighed on gestational days 7 and 20. (Food consumption was not reported.)

On gestational day 20, the rats were sacrificed by CO_2 asphyxiation. The gravid uterus was removed from each animal and weighed. The gravid uteri were examined to determine the numbers of implantation sites, and the numbers of living, dead, and resorbed fetuses. The total weight for all living fetuses for each dam was recorded. Each fetus was examined grossly with the aid of a dissecting microscope for external malformations. Approximately one-half of each litter was fixed in alcohol for skeletal examination; the remainder were fixed in Bouin's fluid for soft tissue examination by the free-hand razor sectioning technique. The sex of each fetus was determined only for the viscerally examined fetuses.

The following statistical analyses were described. Significant differences between the means of observed values among groups were assessed by one-way analysis of variance (ANOVA) for the weight of

live fetuses per litter, initial maternal body weight, corrected final maternal body weights, corrected maternal body weight changes, and percent corrected maternal body weight changes. Number of implantations per litter and live fetuses per litter were analyzed utilizing the non-parametric Kruskal-Wallis procedure. Incidences of fetal anomalies were analyzed by a 2x2 chi-square contingency table and by a "binomial distribution." In all cases, the level of significance was chosen to be $p \leq 0.05$.

9. Results and Discussion:

Prior to discussion of the results of this study, some comments concerning some of its inadequacies are in order. The authors failed to report several important types of data (e.g., number of corpora lutea per dam; maternal food consumption; individual fetal body weights). For other types of data, only minimal observations were made. For example, maternal body weights were measured only at the initiation of dosing and at sacrifice; since virtually all the rats gained weight during the study, the true dosages deviated (decreased) from the target dosages as the animals gained weight. Other problems include: sex was determined in only one-half of the fetuses; the rats were treated beyond the stage of organogenesis (which ends on day 16); according to the authors' method of numbering gestational days (day of confirmed mating = day 1), the rats should have been

killed on day 21, not on day 20. Another unusual feature of this study is that only 20 litters per group were examined teratologically at the terminal sacrifice despite the fact that all groups had more than 20 pregnant dams which survived to term (range 28-37). A total of 51 litters were discarded. These deficiencies weaken any conclusions which are drawn from this study.

Numerous clinical signs were observed in acrolein-treated groups. The incidence of clinical signs increased with dose (low dose = 2; mid dose = 10; high dose = 20). The most common signs included rough hair coat, pilo erection, wheezing, dyspnea, excessive salivation, lethargy and hunched position. Control animals did not exhibit any clinical signs.

Body weight and weight gain data for pregnant rats are presented in Table 1. There were no significant differences among the mean body weights of all groups of animals at the initiation of dosing (day 7); at terminal sacrifice, however, dams in middle- and high-dose groups were significantly lighter than controls ($p \leq 0.001$, ANOVA; $p \leq 0.05$ Duncan's multiple range test). Changes in corrected body weight for both the middle- and high-dose acrolein-treated groups were also significantly smaller than controls (ANOVA, $p \leq 0.01$; Duncan's Multiple range test $p \leq 0.05$). A more accurate assessment of maternal body weight changes in acrolein-treated groups could have been made if the body weights of the pregnant rats had

TABLE 1

BODY WEIGHTS AND WEIGHT GAINS OF PREGNANT SPRAGUE-DAWLEY CD RATS INTUBATED WITH ACROLEIN DURING GESTATIONAL DAYS 7-19[a]

Treatment	No. of Dams	Mean Body Weight at Day 7	Corrected Mean Body Weight at Sacrifice[b]	Change in Corrected Body Weight	
		Grams ± S.D.	Grams ± S.D.	Grams	%
Control	31	210.3 ± 11.2	264.0 ± 13.8[c]	53.6[c]	26
Acrolein (mg/kg)					
3.6	37	206.8 ± 13.5	263.9 ± 16.5[c]	57.2c	28
6.0	35	211.8 ± 10.1	245.0 ± 21.1[c,e]	34.1[c,e]	16
10.0	28	201.9 ± 15.3	216.8 ± 24.3[d,f]	11.7[d,f]	6

[a]Day 1 = day of mating.
[b]Correct Mean Body Weight = Body Weight - Weight of gravid uterus.
[c]n=20.
[d]n=18; The report states that 2 litters were completely resorbed with no rationale why 2 additional litters were not examined from the 10 discarded litters.
[e]Significantly different from control by ANOVA ($p \leq 0.001$); homogeneous subset I by Duncan's multiple range test ($p \leq 0.05$) [Calculated by MITRE].
[f]Significantly different from control by ANOVA ($p \leq 0.001$); homogeneous subset II by Duncan's multiple range test ($p \leq 0.05$) [Calculated by MITRE].

been measured at other times during gestation or if maternal food consumption had been monitored. In the absence of such data, the maternal systemic LEL for acrolein in rats can be estimated to be 6.0 mg/kg, based on decreased body weight gain, the NOEL is 3.6 mg/kg. This assumption is further bolstered by the pattern of maternal mortality in the high-dose group (14/40) and in the mid-dose group (3/40) (see Table 2).

Pertinent gestational data for control and acrolein-treated litters are summarized in Table 2. The mean numbers of implantations per litter were similar among all groups. The pregnancy rates (percent of inseminated dams which were fertilized) of the low- and middle-dose group were significantly greater than the control values ($p \leq 0.05$, Fisher's exact test). This is not treatment related since any changes noted in either the number of pregnant animals or mean number of implantations/litter occurred prior to the initiation of acrolein treatment (which began on gestational day 7), and hence could not be caused by acrolein.

The total number of litters per group which were evaluated in this study was lower than the actual number of pregnant animals. The authors examined only 20 litters per group; the remainder were discarded. The method for selecting the 20 litters which were examined was not described, nor was the rationale for discarding potentially valuable teratologic data given. The conclusions of

TABLE 2

GESTATIONAL DATA FOR SPRAGUE-DAWLEY CD RATS INTUBATED WITH ACROLEIN DURING GESTATIONAL DAYS 7-17

Treatment	No. Fertilized/ No. Inseminated (%)[a]	No. Animals Which Died on Test	No. Litters Evaluated	Implantations		No. Viable Litters with Resorptions
				Total	Mean/Litter	
Control	31/40 (78)	0	20	210	10.5	3
Acrolein (mg/kg)						
3.6	38/40 (93)	1	20	224	11.2	3
6.0	35/40 (88)	3	20	220	11.0	2
10.0	28/40 (70)	14	18[b]	182	10.1	3[b]

[a]Includes animals which died on test.
[b]2 Additional litters were completely resorbed.

any study are strengthened by having a larger sample size, and this remains true in the present instance.

The embryotoxicity data for acrolein are summarized in Table 3. No adverse effects were observed in the numbers or percent of fetal deaths or resorptions; mean number of live fetuses per litter; incidence of anomalous fetuses; or male:female sex ratio of live fetuses. The absence of a reported effect on the male:female sex ratio may not be real since the authors determined the sexed only for those fetuses which were prepared for visceral examination (1/2). This is unusual, since determination of sex in rodents is usually performed at autopsy by grossly visible criteria.

The authors did report a statistically significant increase in anomalous fetuses in all 3 acrolein-treated groups; however, their apparent definition of the term "anomaly" needs to be explained. The authors appear to have defined anomalies according to the scheme discussed by Khera [Fund. Appl. Toxicol. 1:13-18 (1981)] and presented below:

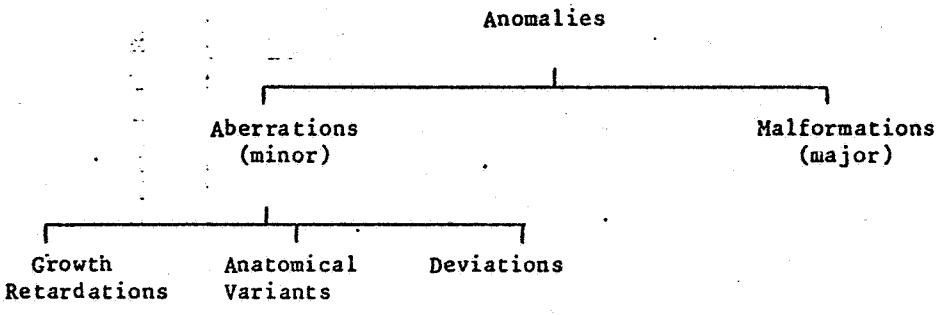

TABLE 3

EMBRYOTOXICITY DATA FOR SPRAGUE-DAWLEY CD RATS INTUBATED WITH ACROLEIN DURING GESTATIONAL DAYS 7-17

Treatment	Total Implantations	Total Resorptions	Dead Fetuses	Percent Dead or Resorbed	Live Fetuses		Mean Fetal Weight (gm)	No. of Anomalous Fetuses (%)	Male/Femal Ratio of Live Fetus (% Male)
					Total	Mean/ Litter			
Control	210	4	0	1.9	206	10.3	2.4	62 (30)	44/63 (4
Acrolein (mg/kg)									
3.6	224	2	4	2.7	218	11.0	2.6	26 (12)	60/53 (5
6.0	220	2	1	1.4	217	10.9	2.7	41 (19)	58/52 (5
10.0	182	5	3	4.4	174	9.7	1.9	68 (39)	36/55 (4

[a]The sex was determined for only fetuses subjected to visceral examinations (=1/2).

In this scheme, "anomalies" include all major and minor anatomical changes, although only major malformations are indicators of teratogenicity. Although the authors recognized the fact that many of their findings were, in fact, only minor anatomical changes, they did not differentiate major and minor changes when performing their statistics. Tables 4 and 5 were prepared by MITRE to dichotomize anomalies into major malformations, which are life-threatening and provide evidence of teratogenicity (Table 4); and aberrations, which are changes of a minor nature (Table 5). Due to the lack of descriptions for individual fetuses, it was not always possible for MITRE to determine whether 2 or more anomalies occurred in a single fetus.

The incidence of major malformations is presented in Table 4. The high-dose group had significantly more malformed fetuses than controls ($p \leq 0.01$, Fisher's exact test). The majority of the malformed fetuses in the high-dose group were runts. The authors described these fetuses as exhibiting "retarded development of the entire fetus." Since that is a subjective description, MITRE classified fetuses as "runts" if their weights were more than 2 standard deviations below the mean control fetal weights. According to the original report, the controls for this study weighed 2.4 ± 0.6 g; thus, all fetuses weighing less than 1.2 g were classified as runts.

TABLE 4

INCIDENCE OF RAT FETUSES EXHIBITING MAJOR MALFORMATIONS AFTER MATERNAL INTUBATION WITH ACROLEIN DURING GESTATIONAL DAYS 7-19

Treatment	Anophthalmia	Microphthalmia	Micrognathia	Runt	Total
Control	1/108	0/108	1/108	0/106	2/108
Acrolein (mg/kg)					
3.6	0/113	0/113	0/113	0/113	0/113
6.0	0/110	0/111[a]	0/110	0/110	0/110[d]
10.0	2/94[b]	1/94[b]	0/04[b]	8/95[c]	11/94[d,e]

[a]According to the original reports, a total of 111 fetuses were scored for microphthalmia; 110 for other external and all visceral malformations.
[b]According to the original report, a total of 94 fetuses were scored for anopthalmia, microphthalmia, and macrognathia; 91 for visceral malformations.
[c]According to the original report, a total of 95 fetuses were scored for being a fetal runt; 91 for visceral malformations.
[d]To be conservative, the denominator for total incidence is the smallest denominator for a given treatment group.
[e]Significantly greater than control $p \leq 0.01$ (Fisher's Exact Test) (Calculated by MITRE).

TABLE 5

INCIDENCE OF RAT FETUSES EXHIBITING ANATOMICAL ABERRATIONS AFTER MATERNAL INTUBATION WITH ACROLEIN DURING GESTATIONAL DAYS 7-19

Treatment	Dilated Renal Pelves and/or Ureter	Malaligned Kidney	Incomplete Ossification						No. Fetuses with at Least one Skeletal Aberration
			Skull	Sternum	Pelvic Girdle	Vertebrae	Meta-carpals	Meta-tarsals	
Control	7/108	0/108	0/97	20/97	51/97	5/97	2/97	19/97	55/97
Acrolein (mg/kg)									
3.6	8/113	0/113	0/107	2/107	11/107	0/107	4/107	12/107	18/107
6.0	8/110	3/110	1/107	9/107	26/107	8/107	6/107	10/107	30/107
10.0	2/91	0/91	9/83	29/83	33/83	21/83	22/83	34/83	62/83

The standard deviations in the original reports were not included in this data evaluation record because they were calculated incorrectly. The authors recorded only the entire weight of a litter and divided that weight by the number of pups in the litter (as opposed to weighing fetuses individually). Thus, the standard deviations which they reported for the mean fetal weights in the original report do reflect the true variance in the sample size and are too small. MITRE used the "small standard deviation" in its definition of runts because a smaller standard deviation around the mean control fetal weight would increase the number of pups which fall outside the working definition of a "runt" (mean - 2 S.D.). This means that the data presented in this DER are conservative.

It should be noted that although 8/95 high dose pups were classified as runts, they all came from one litter. All of the members of that litter were runts. The dam which bore that litter lost weight during her pregnancy (i.e., she had a negative weight gain); but other dams also lost weight with no ill-effects seen on their pups. Another possible explanation for a litter of runts is that the pregnancy was mistimed. If the dam was not actually shipped from the breeding facility on gestational day 5, but rather shipped on day 3, a litter of normal, day 18 pups would appear to be runts if the investigators thought the pups were at gestational day 20.

The incidences of minor fetal aberrations are summarized in Table 5. There were numerous fetuses exhibiting aberrations,

especially in the control and high-dose acrolein group. Dilated renal pelves are anatomical variants; delayed ossifications are growth retardations. The authors do not describe "malaligned kidneys." They may be either anatomical variants or deviations.

The vast majority of fetal aberrations in both control and treated groups were delayed ossifications in the skeletal system. The apparent increase in skeletal delays may be due to premature sacrifice of the animals (1 day). The usual convention for timing pregnancy in rodents is to designate the data of confirmation of pregnancy as day 0; rats are sacrificed on day 20 using that convention. The authors of the present report designated the confirmation day as day 1; by the authors' method of counting gestational days, terminal sacrifice should have taken place on day 21, not on day 20. Although the changes noted may be due to premature sacrifice, it should be pointed out that the high-dose group exhibited increased delays in ossification of the skull, vertebrae, metacarpals, and metatarsals when compared to controls.

Thus, acrolein does induce malformations (runts) in rats at 10 mg/kg, but that dosage is quite toxic to the mothers (reduced maternal body weight gains and increased mortality [14/40]). At the mid-dose level (6.0 mg/kg), maternal toxicity was still present (reduced maternal body weight gains) but there were no malformations

or apparent fetotoxicity. Therefore, the teratogenic and fetotoxic LELs are estimated to be 10 mg/kg/day; the NOELs are 6.0 mg/kg/day. Both are above the NOEL for maternal toxicity.

Two additional discrepancies in the subject report should also be noted.

[redacted]

- The reference given for skeletal staining (Inouye 1976) describes a method for combining staining of cartilage and bone. Did the authors in fact perform dual staining? If so, many of their putative aberrations could be more accurately scored. If they did not perform dual staining, what method did they use?

10. Technical Review Time: 36 hours.

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* CONFIDENTIAL BUSINESS INFORMATION *

MRID: Not assigned

1. Chemical or Chemicals:

Acrolein

2. Type or Formulation:

Technical (> 96%; 4% unspecified)

3. Citation or Citations:

King, M. and J.A. Salinas "Teratology study of acrolein in mice" Bioassay Systems Corporation Project No. 10258, submitted by Magna Corporation, Houston, Texas, 1 September 1982

4. Reviewed by:

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Signature: John M. DeSesso
Date: 3/4/83

5. Approved by:

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Toxicology Branch (TS-769)
Hazard Evaluation Division
Office of Pesticides and Toxic Substances
U.S. Environmental Protection Agency

Signature: Chad B. Sandusky
Date: 3/4/83

6. Discipline/Topic or Test Type:

This study has information pertinent to discipline toxicology, TOPIC TERATOGENICITY.

This study relates to the Proposed Guidelines data requirement 163.83-3.

* CONFIDENTIAL BUSINESS INFORMATION *

7.0 Conclusions:

In this teratology study, technical grade acrolein (>96% pure) was diluted with deionized water and administered by gastric intubation to mated, female CD-1 mice (32/group) at dosages of 4.0, 6.3, or 10.0 mg/kg on gestational days 7-17. Control dams received deionized water only. At terminal sacrifice, the body weight gains of dams in all 3 acrolein-treated groups were significantly smaller than controls (see Tables 1 and 2). Based on these data, a maternal systemic NOEL for acrolein in pregnant mice was not established (<4 mg/kg).

No ill effects were observed in fetal viability, fetal weights, or male:female sex ratios. The incidences of fetuses exhibiting at least 1 anomaly (see Table 3) were higher in all 3 treated groups than in the control group. The preponderance of anomalies was due to minor fetal aberrations (see Table 5; dilated renal pelves; delayed ossifications of sternebrae, metacarpals or metatarsals; subcutaneous edema; 14th rib). The overall incidence of major malformations (see Table 4) did not differ among all 4 groups; however, 4 cases of cleft palate occurred in the acrolein-treated groups (2 each in the mid- and high-dose groups).

As discussed below, the paucity of critical, teratologic data precludes drawing conclusions concerning the teratogenicity of

acrolein in mice from this study at this time. It remains unclear if cleft palate is a possible teratogenic effect of acrolein. (Historical data is requested, see page 17.) However, acrolein was demonstrated to be embryotoxic at doses as low as 4 mg/kg (Lowest Dose Tested) based upon the overall increased incidence of both major and minor anomalies among all acrolein-treated groups. Therefore, a fetotoxic NOEL was not established in this study.

CORE CLASSIFICATION: Supplementary. This study exhibited numerous short-comings:

- too few pregnant animals were analyzed in this study (range 12-20 litters/group).
- the complete description of the chemical tested was not given; 4% unspecified
- several important types of data were not reported
  - maternal food consumption
  - numbers of corpora lutea
  - individual fetal body weights
- only one-half of the fetuses were examined for cleft palate. The registrant should be requested to examine all skeletal preparations for the presence or absence of clefts of the hard palate.
- maternal toxicity was observed at all doses, even the lowest, therefore a NOEL for maternal toxicity was not established.
- fetotoxicity (generalized delayed ossification of fetuses) was observed at lowest dose tested, therefore the fetotoxicity NOEL was not established.
- the animals were treated beyond the stage of organogenesis; dosing should have been stopped at day 16.

- the animals were killed too early; they should have been sacrificed on day 19 vice day 18.
- sex was determined in only one-half of the fetuses.
- maternal body weights were measured only on the first day of dosing and at terminal sacrifice.

8. Materials and Methods:

Technical grade Acrolein (CAS No. 000107028; purity >96%) was obtained from Magna Corporation, Santa Fe Springs, CA. The material was a colorless liquid which was stored in a sealed container under argon at room temperature. The complete chemical composition of the test material was not supplied by Magna Corporation.

A total of 128 timed-pregnant CD-1 mice were purchased from Charles River Breeding Laboratories, Wilmington, Massachusetts. The animals arrived at the testing facilities of Bioassay Systems Corporation on gestational day 5; the day that mating was confirmed was designated day 1 of gestation. Neither details of the mating procedures nor the means for confirmation of pregnancy were available. Upon arrival, the mice were 7-9 weeks old and weighed 22-34 g. They were identified by animal numbers which were marked by an ear punch method. Each mouse was housed individually in polycarbonate cages containing hardwood chip bedding (Sani-chips) in a climate controlled animal facility. Ambient temperature was maintained at 20-21°C; the relative humidity was 47-56%; and the

light/dark cycle allowed 12 hours of light per day. Air flow in the animal quarters varied from 12-16 complete changes of charcoal filtered fresh air each hour. Throughout the study, mice were allowed free access to Charles River Pelleted Rodent Diet and tap water.

Upon receipt on gestational day 5, the animals were quarantined for 2 days and observed daily for signs of disease. On gestational day 7, the mice were assigned to one of the 4 treatment groups (32 mice/group) by means of a random numbers table. The experimental group and dosage level were recorded on each animal's cage card.

The mice received daily doses of the test compound by gastric intubation on gestational days 7-17. Based upon each mouse's body weight on gestational day 7, test dosages of 4.0, 6.3, or 10.0 mg/kg of acrolein were dissolved in sufficient deionized water to ensure that the volume of each dose was 10 ml/kg body weight. Formulations of stock treatment solutions were stored in the dark under argon at room temperature for not more then 5 days. Fresh stock solutions were prepared 3 times during the study. Control animals received deionized water only.

During the period of testing, the mice were observed twice daily for clinical signs. Each mouse was weighed on gestational days 7 and 18. (Food consumption was not reported.) During the seventh day on

test (i.e. gestational day 14), 2 animals per group were removed from the test and evaluated for health status.

On gestational day 18, the mice were sacrificed by CO_2 asphyxiation. The gravid uterus was removed from each animal and weighed. The gravid uteri were examined to determine the numbers of implantation sites, and the numbers of living, dead, and resorbed fetuses. The total weight for all living fetuses for each dam was recorded. Each fetus was examined grossly with the aid of a dissecting microscope for external malformations. Approximately one-half of each litter was fixed in alcohol for skeletal examination; the remainder were fixed in Bouin's fluid for soft tissue examination by the free-hand razor sectioning technique. The sex of each fetus was determined only for the viscerally examined fetuses.

The following statistical analyses were described. Significant differences between the means of observed values among groups were assessed by one-way analysis of variance (ANOVA) for the weight of live fetuses per litter, initial maternal body weight, corrected final maternal body weights, corrected maternal body weight changes, and percent corrected maternal body weight changes. Numbers of implantations per litter and of live fetuses per litter were analyzed utilizing the non-parametric Kruskal-Wallis procedure. Incidences of

fetal anomalies were analyzed by a 2x2 chi-square contingency table and by a "binomial distribution." In all cases, the level of significance was chosen to be $p \leq 0.05$.

9. Results and Discussion:

Prior to discussing the results of this study, some comments concerning its general inadequacy are in order. Too few pregnant animals (n=12-20) were present in each group (except the middle-dose group receiving 6.3 mg/kg). The authors failed to report several important types of data (e.g., number of corpora lutea per dam; maternal food consumption; individual fetal body weights). For other types of data, only minimal observations were made. For example, maternal body weights were measured only at the initiation of dosing and at sacrifice; since the pregnant mice virtually all gained weight during the study, this means that the true dosages deviated from the target dosages by decreasing as the mice gained weight. Other problems include the following: sex was determined for only one-half of the fetuses; only one-half of the fetuses were examined for cleft palate; the mice were treated beyond the stage of organogenesis (which ends on day 16); by the authors' method of numbering gestational days (day of mating = day 1), the mice should have been killed on day 19, not on day 18. These data gaps weaken any conclusions which are drawn from this study.

Clinical signs were observed in all experimental groups. Rough haircoat was observed with about equal frequency in all groups including controls. Lethargy was observed more frequently among treated mice than controls, especially in the high-dose group. Other signs which appeared more frequently among treated mice were squinted eyes, "apparent weight loss" (body weights were measured only at sacrifice), and hunched posture.

Body weight and weight gain data for pregnant mice are presented in Table 1. There were no significant differences among the mean body weights of all groups of animals either at the initiation of dosing (day 7) or at sacrifice. Changes in corrected body weight among all 3 acrolein-treated groups were significantly smaller than controls (ANOVA, $p \leq 0.01$; Duncan's Multiple range test $p \leq 0.05$). This finding disagrees with the authors' report, which states that the significant effect on body weight changes was "attributed to a small weight gain (2.6 ± 1.9) among animals from the 10.0 mg/kg dose group, as compared to the control group (4.9 ± 1.6)." The authors' statement implies that the body weight change of the high-dose group differed from the other 3 groups. Although the authors used the appropriate statistical procedure (ANOVA) to determine that the means of the 4 groups differed, they did not use an appropriate statistical procedure to identify which means differ from each other. Using the

TABLE 1

BODY WEIGHTS AND WEIGHT GAINS OF PREGNANT CD-1 MICE INTUBATED WITH ACROLEIN DURING GESTATIONAL DAYS 7-17[a]

Treatment	No. of Dams	Mean Body Weight at Day 7	Corrected Mean Body Weight at Sacrifice[b]	Change in Corrected Body Weight	
		Grams ± S.D.	Grams ± S.D.	Grams	%
Control	16	27.1 ± 2.2	32.0 ± 2.7[c]	4.9[c]	18
Acrolein (mg/kg)					
4.0	18	27.4 ± 2.6	31.0 ± 2.3[d]	3.6[d,f]	13
6.3	20	26.6 ± 1.9	30.1 ± 2.1	3.5[f]	13
10.0	13	28.2 ± 2.5	31.3 ± 1.7[e]	2.6[e,f]	9

[a]Day 1 = day of mating.
[b]Corrected Mean Body Weight = Body Weight - Weight of gravid uterus.
[c]n=15; gravid uterus for one mouse not weighed.
[d]n=17; gravid uterus for one mouse not weighed.
[e]n=12; one animal died on test.
[f]Significantly different from control by ANOVA ($p \leq 0.01$); homogeneous subset by Duncan's multiple range test ($p \leq 0.05$) [Calculated by MITRE].

Duncan's Multiple range test demonstrates that it is the control mean which differed from the 3 treated means (performed by MITRE). Thus, all 3 acrolein-treated groups displayed significantly decreased body weight changes at term ($p \leq 0.05$).

A more accurate indication of whether the smaller body weight changes in acrolein-treated groups are clear signs of maternal toxicity could have been made if the body weights of the pregnant mice had been measured at other times during gestation and if maternal food consumption had been monitored. In the absence of such data, the conservative approach of considering the smaller body changes in acrolein-treated mice to be signs of maternal toxicity is appropriate. Thus, the maternal systemic NOEL for acrolein in mice was not determined by this study, since all treated dams had decreased body weight changes at sacrifice when compared to controls.

Pertinent gestational data for control and acrolein-treated litters are summarized in Table 2. Acrolein caused no statistically significant adverse effects in any of the parameters listed. These values demonstrate the homogeneity of the experimental system at the beginning of the test. If any changes had been noted in the number of pregnant animals or mean number of implantations/litter, they would have occurred prior to the initiation of acrolein treatment (which began on gestational day 7), and hence could not be caused by acrolein.

TABLE 2

GESTATIONAL DATA FOR CD-1 MICE INTUBATED WITH ACROLEIN DURING GESTATIONAL DAYS 7-17

Treatment	No. Fertilized/ No. Inseminated (%)[a]	No. Litters/ No. Inseminated (%)	Implantations		No. Litters with Resorptions
			Total	Mean/Litter	
Control	18/32 (56)	16/30 (53)	170	10.6	3[c]
Acrolein (mg/kg)					
4.0	19/32 (59)	18/30 (60)	191	10.6	4[d]
6.3	21/32 (66)	20/30 (67)	212	10.6	3[d]
10.0	15/32 (47)	12/29[b] (41)	131	10.9	5[c]

[a]Includes animals removed from the test on gestational day 14 for health status evaluation and fertilized animals which completely resorbed the products of conception at an early stage, detected only after sodium sulfide staining.
[b]One pregnant animal died on test.
[c]2 Additional Litters were completely resorbed (early resorptions).
[d]1 Additional litter was completely resorbed (early resorptions).

The total number of pregnant animals in this study was low (only 73/128 or 57%). This may have been due to preimplantation losses which can be caused by maternal stress suffered during shipping. Another explanation could be misdiagnoses of pregnancies. The latter situation would not be a result of the authors' misdiagnoses, but rather that of the animal supplier who provided "timed-pregnant" animals. In any case, the number of pregnant animals per group in this study is unsatisfactory for good experimental design in a teratology study.

The embryotoxicity data for acrolein are summarized in Table 3. No adverse effects were observed in the numbers of percent of fetal deaths or resorptions; mean number of live fetuses per litter; mean fetal weights; or male:female sex ratio of live fetuses. The absence of effect on the male:female sex ratio must be viewed with caution since the authors determined the sex in only those fetuses prepared for visceral examination. This is unusual, since determination of sex in rodents is usually performed at autopsy by grossly visible criteria.

The authors did report a statistically significant increase in anomalous fetuses in all 3 acrolein-treated groups; however, their apparent definition of the term "anomaly" needs to be explained. The authors appear to have defined anomalies according to the scheme

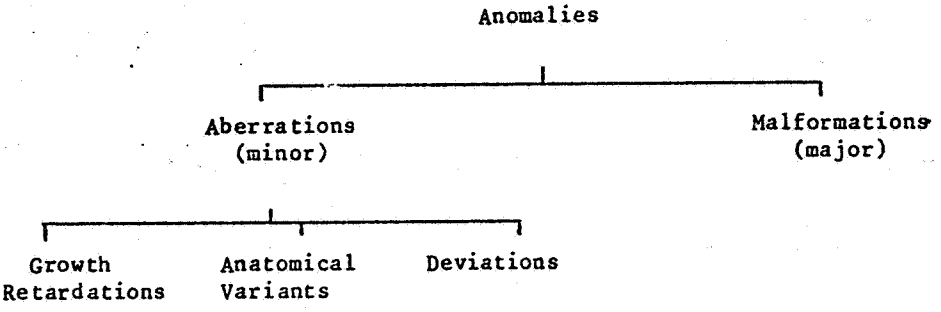
TABLE 3

EMBRYOTOXICITY DATA FOR CD-1 MICE INTUBATED WITH ACROLEIN DURING GESTATIONAL DAYS 7-17

Treatment	Total Implantations	Total Resorptions	Dead Fetuses	Percent Dead or Resorbed	Live Fetuses		Mean Fetal Weight (gm)	No. of Anomalous Fetuses (%)	Male/Female Ratio of Live Fetuses[a] (% Male)
					Total	Mean/ Litter			
Control	170	1	2	1.8	167	10.4	1.12	21[a] (13)	37/43 (46)
Acrolein (mg/kg)									
4.0	191	4	3	3.7	184	10.2	1.15	48[a,c] (26)	37/50 (43)
6.3	212	2	2	1.9	207	10.4	1.13	88[a,d] (43)	46/53 (46)
10.0	131	4	3	5.3	124	10.3	1.08	68[a,d] (55)	30/28[e] (52)

[a]Only fetuses subjected to visceral examinations were sexed (≈1/2).
[b]Significantly different from control, $p \leq 0.0001$ (Chi-square, 3 degrees of freedom) (Calculated by MITRE).
[c]Significantly different from control, $p \leq 0.001$ (Fisher's Exact Test, 1 tail) (Calculated by MITRE).
[d]Significantly different from control, $p \leq 0.0001$ (Fisher's Exact Test, 1 tail) (Calculated by MITRE).
[e]Sex not determined for one fetus.

discussed by Khera [Fund. Appl. Toxicol. 1:13-18 (1981)] and presented below:



In this scheme, "anomalies" include all major and minor anatomical changes, although only major malformations are indicators of teratogenicity. Although the authors recognized the fact that many of their findings were, in fact, only minor anatomical changes, they did not differentiate major and minor changes when performing their statistics. Tables 4 and 5 were prepared by MITRE to dichotomize anomalies into major malformations, which are life-threatening and provide evidence of teratogenicity (Table 4); and aberrations, which are changes of a minor nature (Table 5). Due to the lack of descriptions for individual fetuses, it was not always possible for MITRE to determine whether 2 or more anomalies occurred in a single fetus.

The total incidence of major malformations (Table 4) is similar among all 4 groups. Cleft palates occurred in a total of 4 fetuses

TABLE 4

INCIDENCE OF FETUSES EXHIBITING MAJOR MALFORMATIONS AFTER MATERNAL INTUBATION WITH ACROLEIN DURING GESTATIONAL DAYS 7-17

Treatment	Cleft Palate	Exencephaly	Microphthalmia	Umbilical Hernia	Total Major Malformations
Control	0/80	0/80	1/80	0/80	1/80
Acrolein (mg/kg)					
4.0	0/87	1/87	0/87	1/87	2/87
6.3	2/99	0/99	0/99	0/99	2/99
10.0	2/59	0/59	0/59	0/59	2/59

TABLE 5

INCIDENCE OF FETUSES EXHIBITING ANATOMICAL ABERRATIONS AFTER MATERNAL INTUBATION WITH ACROLEIN DURING GESTATIONAL DAYS 7-17

Treatment	Subcutaneous Edema (%)	Dilated Renal Pelvis (%)	14th Rib (%)	Delayed Ossification			
				"Generalized" (%)	Metacarpals (%)	Metatarsals (%)	Sternebrae (%)
Control	0/80 (0)	5/80 (6)	1/87 (1)	6/87 (7)	0/87 (0)	2/87 (23)	0/87 (0)
Acrolein (mg/kg)							
4.0	0/87 (0)	7/87 (8)	2/97 (2)	17/97 (18)	4/97 (4)	9/97 (9)	0/97 (0)
6.3	2/99 (2)	31/99 (31)	2/108 (2)	22/108 (20)	2/108 (2)	2/108 (2)	0/108 (0)
10.0	19/59 (32)	16/59 (27)	4/65 (62)	13/65 (20)	8/65 (12)	10/65 (15)	5/65 (8)

(2 in each of the middle- and high-dose groups); however, cleft palate (a grossly observable major anomaly), was looked for only in those fetuses which were examined for visceral malformations. The incidence of cleft palate in 2 higher dose groups is the pivotal finding of this study. Did any controls prepared for skeletal examination exhibit cleft palate? Were additional clefts of the hard palate to be found among the skeletal preparations of other acrolein-treated fetuses? Without these data, it is difficult to make a scientific judgment concerning possible teratogenicity. The incidence of cleft palate in the mid- and high-dose groups is a biological significant effect which cannot be properly evaluated without a larger sample size. Therefore, a teratogenic NOEL was not established and the cleft palate remains a possible teratogenic effect of acrolein in mice.

In order to provide a historical perspective to the incidence of malformations in CD-1 mice, the authors included in their report a copy of a paper by Perraud which documented the spontaneous occurrence of malformations in CD-1 mice. Only the odd-numbered pages of the article were included, however, and the data relating to cleft palates were tabulated on an even-numbered page. Thus, the necessary historical data were not available for review and should be requested from the registrant.

The incidences of fetal aberrations are presented in Table 5. Fetuses from acrolein-treated mothers exhibited more aberrations than controls, especially in the high-dose group. Delayed ossifications fall under the classification of growth retardations; dilated renal pelves are anatomical variants; 14th ribs are deviations. The biological significance of subcutaneous edema is not known. The authors did not attempt to score the severity of edema. In addition to the aberrations listed in Table 5, the authors reported the following apparent incidences of fetal hemmorhaging or fragile vessels in the control and treated groups, respectively: 1/80, 8/87, 35/99, and 19/59. As in the case of subcutaneous edema, the significance of these findings is not known.

Based on the above data (Table 5), a conservative interpretation of the data in the subject report is that acrolein is embryotoxic at levels as low as 4 mg/kg (Lowest Dose Tested) (based on increased incidences of fetal aberrations). Therefore, a fetotoxic NOEL was not established. In addition, no statements regarding teratogenicity of acrolein can be made from the data in this study for the following reasons:

- too few pregnant mice were employed
- cleft palates were scored in only one-half of the fetuses
- maternal toxicity was observed at all dosages

It is recommended that the authors be requested to retrieve the specimens from their archives and examine all skeletal preparations, for the presence or absence of clefts of the hard palate. Upon submission of such data, as well as historical control data, the subject study should be re-evaluated. Although the numbers of pregnant animals in this study are low, the verification of acrolein-induced cleft palate in CD-1 mice in the subject study is important.

Several additional discrepancies in the subject report should also be noted.

[redacted]

- The reference given for skeletal staining (Inouye 1976) describes a method for combining staining of cartilage and bone. Did the authors in fact perform dual staining? If so, many of their putative aberrations could be more accurately scored. If they did not perform dual staining, what method did they use?

- In the 6.3 mg/kg group, the authors report 2/99 cleft palates ($p \leq 0.05$) and 2/99 cases of subcutaneous edema ($p \leq 0.01$). Since the incidence in controls for both anomalies was 0/80, one of the p-values is wrong and should be corrected.

10. Technical Review Time: 43 hours.