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DER #1

Isocyanuric acid: Developmental Toxicity in the Rabbit.
Isocyanurate Industry ad hoc Committee, 1990
MRID No. 42054101
HED Doc. No. 009563

PC 08140Y

Primary Review by: Steven L. Malish, Ph.D. S.L. Malish 5/11/92
Review Section IV, Toxicology Branch II (H7509C)
Secondary Review by: Elizabeth A. Doyle, Ph.D. E.A. Doyle 5/18/92
Section Head, Review Section IV, Toxicology Branch II (H7509C)

DATA EVALUATION RECORD 009503

Study Type: Developmental Toxicity Study - rabbit (83-3)

MRID No.: 420541-01

Test Material: Isocyanurate, monosodium isocyanurate

Synonyms: Sodium cyanurate

Sponsor: Isocyanurate Industry ad hoc Committee
800 N Lindbergh Blvd. G5NC
St. Louis, MO 63167

Study Number: 3222.1

Testing Facility: Springborn Laboratories, Inc.
Life Sciences Division
553 North Broadway
Spencerville, OH 45887

Title of Report: Teratology Study in Rabbits with Monosodium Isocyanurate

Author: Dean E. Rodwell, M.S.

Report Issued: December 4, 1990

Conclusions:

Monosodium Isocyanurate was administered to pregnant New Zealand White rabbits as a single daily dose by gavage at dose levels of 0 (Control), 50, 200 and 500 mg/kg/day from day 6 to 19 of gestation.

Maternal toxicity was not observed during the study.

A decrease in the mean number of viable fetuses was noted at the 500 mg/kg/day dose level when compared to the control group. This was caused by an increase in late resorptions and reflected in the mean post-implantation loss incidence of 21.9% versus the control incidence of 7.3%.

Comparison of the fetal data to their respective controls indicated that the 500 mg/kg/day dose had a 12.2% incidence of hydrocephaly versus 2.3% in the controls. The incidence of hydrocephaly showed a dose related trend when the litter data was analyzed.

NOEL (maternal) = 500 mg/kg/day (HDT)
LOEL (maternal) = not established; >500 mg/kg/day
NOEL (developmental) = 200 mg/kg/day
LOEL (developmental) = 500 mg/kg/day (hydrocephaly, post-implantation loss)

Classification: Core - guideline

This study satisfies the guideline requirement 83-3 for a developmental toxicity study.

Quality Assurance - A signed and dated quality assurance statement was provided.

Flagging Criteria:

A signed and dated flagging statement was included on p. 4 of the original report that noted that "this study neither meets or exceeds any of the applicable [flagging] criteria." The reviewer agrees with this conclusion.

A. Materials and Methods

Test Compound:

Chemical:	Monosodium Isocyanurate
Purity:	>99%
Description:	white amorphous powder
Lot No.:	#NB:4,409,462
Manufacturer:	Monsanto Company, St. Louis, MO.
Storage:	Refrigerated
Stability:	Stable under the test conditions

Vehicle:

Chemical:	1% w/v methylcellulose aqueous solution
Source:	Fisher Scientific, Cincinnati, OH
Lot No.:	874544
Stability:	Stable under the test conditions

Dosing Solution Preparation

A mortar and pestle was used to grind the test compound which was then sieved through a 40 mesh screen. The material was weighed into a calibrated beaker and a small amount of the vehicle was added with stirring to suspend the active ingredient; an additional quantity of vehicle was then added to achieve the desired concentrations.

A magnetic stirrer was used to produce a homogeneous dosing suspension. Each suspension was stirred for 30 minutes prior to and continuously during dosing. Suspensions were prepared each week and refrigerated.

Formulation Attributes

Stability

Sample suspensions were considered to be stable as evidenced by mean recoveries of the 25 mg/ml and 250 mg/ml dosing suspensions that were within 3% of the nominal concentration for up to 7 days after preparation.

Concentration

The mean recoveries of the 0, 25, 100 and 250 mg/ml suspensions were within 10% of the nominal concentrations when analyzed the first day of dosing and near the end of the dosing period.

Homogeneity

The sample suspensions were considered to be homogenous and within 3% of the nominal concentrations for the mean recoveries for the upper, middle and lower portions of the sample suspensions.

Statistical Analysis

According to the report 3222.1 p. 52 "All analyses were two-tailed with a minimum significance level of 5%. One way analysis of variance followed by a Dunnett's test was used to analyze maternal and fetal data including body weights, food consumption, number of viable fetuses, implantation sites and corpora lutea. The Mann-Whitney U test was used to compare post-implantation loss, dead fetuses and resorptions. Fetal sex ratios were analyzed using the Chi-Square test. Fisher's Exact test was used to analyze the incidence and number of fetal malformations and variations utilizing the dam (litter) as the experimental unit". The Exact Trend test was used to analyze the litter hydrocephaly data. Historical control data were provided to allow comparison with concurrent controls (p. 221-223 of the original report).

Test Animals:

Species:	rabbit
Strain:	New Zealand White
Sex:	female
Groups:	4 groups of 20 animals each
Age:	5 to 7 months
Weight:	3 to 5 kg
Source:	Hazleton Research Products, Inc. Denver, PA

Acclimatization and Housing

Animals were acclimated for a minimum of 28 days before being placed on test.

The animals were individually housed in suspended cages. Purina Certified Rabbit Chow #5322 and deionized tap water were provided ad libitum throughout the study.

Study Design

This study was designed to assess the developmental toxicity potential of the test material, monosodium isocyanurate, when administered by a single oral dose by gavage to rabbits on gestation days 6 to 18 inclusive.

Eighty (80) virgin female New Zealand White rabbits were assigned to control or treatment groups by the use of a stratified body weight randomization method (Table 1).

Table 1

Group Treatments[1]

Test Group	Dose (mg/kg)	Conc. (mg/ml)	Vol. (ml/kg)	No.
Control	0	0	2	20
Low Dose	50	25	2	20
Mid Dose	200	100	2	20
High Dose	500	250	2	20

[1]Adapted from original report, p. 13.

Mating

Semen from New Zealand White rabbits, obtained from the same source as the females, was evaluated for volume, motility and concentration. During the insemination procedure, the semen was diluted with 0.9% saline and maintained in a water bath at 36° C.

The day of insemination was considered to be day 0 of gestation. Semen from one male was used to inseminate an equal number of females in each study group. Approximately 0.5 ml of the diluted semen was introduced into the vagina of each animal. After insemination, females were administered human chorionic gonadotropin by the marginal ear vein.

Observations

All animals were observed daily for signs of toxicity. During the treatment period, the rabbits were observed between 0.5 to 2 hours

after dosing for treatment related effects. Mortality checks were performed twice a day.

Observation of the control and treated animals were considered to be not remarkable.

Body Weight

Animals were weighed on gestation days 0, 6, 9, 12, 15, 19, 24 and 29. Body weight changes were calculated for the following gestation times: 0-6, 6-9, 9-12, 12-15, 15-19, 19-24, 24-29, 6-19, 19-29 and 0-29 days.

Food Consumption

Food consumption was measured each day during gestation and calculations were made for gestation days 0-6, 6-9, 9-12, 12-15, 15-19, 19-24, 24-29, 6-19, 19-29 and 0-29.

Sacrifice

Females that aborted during the study were sacrificed. Body cavities were opened and examined. The number of corpora lutea on each ovary was counted. Uterine contents were examined and the implants recorded. Abnormalities were recorded.

Surviving dams were sacrificed on day 29 of gestation and subjected to examination similar to the above.

Cesarean Section Observations

According to the original report, p. 15, "the uterus was removed from the body, examined externally, weighed and then opened for an internal examination. The number of viable and non-viable fetuses and early and late resorptions was recorded beginning with the left distal horn, noting the position of the cervix and continuing to the right distal horn. Corpora lutea were counted for each ovary. Uteri with no macroscopic evidence of implants were placed in a 10% aqueous ammonium sulfide solution for detection of early embryolethality."

Fetal Morphological Evaluations

External examination

The fetuses were weighed and individually identified. The crown rump length of late resorptions was measured. Each fetus was examined for external abnormalities.

Visceral Examination

Each fetus was dissected and examined under low powered magnification. During the procedure, the sex of the fetus was determined.

Skeletal Examination

Fetuses were eviscerated, skinned and fixed in 95% isopropyl alcohol. Fetuses were then macerated in 1.5% aqueous potassium hydroxide solution, stained with alizarin Red S and cleared in 25% aqueous glycerin solution. Skeletal examinations were performed using substage lighting.

B. Results

Maternal Toxicity

No maternal treatment related clinical signs of toxicity were observed during the study.

No statistically significant differences were noted in the mean body weight and body weight change values between the control and treated groups although considerable variation was noted between the groups (Table 2).

A slight reduction in the body weight gain and a slight weight loss occurred at the 500 mg/kg/day dose levels during gestation days 12-15 and 15-19, respectively. At the 200 mg/kg/day dose level, a minimal body weight loss occurred during gestation days 15-19 (Table 2).

Table 2

Gestation Body Weight Gain[1,2]

	Mean Body Weight Gain (gm) (mg/kg/day)			
Period	0	50	200	500
Day 0-6	272	284	249	289
Day 12-15	60	67	64	25
Day 15-19	19	41	-12	-32
Day 6-19	171	211	120	107
Day 19-29	105	36	12	190
Day 0-29	548	555	380	573
Day 0-29[3]	95	69	8	167

[1]Adapted from original report, p. 26-27.
[2]Values were not statistically significant.
[3]Corrected values

A slight dose related decrease in food consumption occurred during gestation days 15-19 at 200 and 500 mg/kg/day. No significant differences were noted in the mean food consumption values between the control and treated groups although considerable variation was noted between the groups
(Table 3).

Table 3

Mean Food Consumption Data (gm/animal/day)[1,2]

Time Period	Dose Level (mg/kg/day) 0	50	200	500
Day 0-6	186	188	171	193
Day 12-15	160	172	153	156
Day 15-19	166	178	147	135
Day 6-19	178	187	164	168
Day 19-29	136	130	117	150
Day 0-29	165	169	149	164

[1]Adapted from original report, p. 28-29.
[2]Values were not statistically significant.

A single female at the 500 mg/kg/day level aborted on gestation day 22; 1 female each at the 50 mg/kg/day group aborted on gestation days 24 and 26. All the other females in the control and treated group survived to the scheduled sacrifice (day 29). The pregnancy rate in the control and 200 mg/kg/day group was 90% versus 100% in the 50 mg/kg/day groups and 80% in the 500 mg/kg/day group
(Table 4).

Table 4

Survival and Pregnancy Parameters in Rabbits Treated with Monosodium Isocyanurate[1,2]

	Dose (mg/kg/day)			
	0	50	200	500
Females on Study:	20	20	20	20
Parameter (%)				
Females Aborted	0 (0)	2 (10)	0 (0)	1 (5)
Nongravid	2 (10)	0 (0)	2(10)	4 (21.1)
Gravid	18 (90)	18 (100)	18 (90)	15 (78.9)
with resorptions only	0 (0)	0 (0)	1 (5.6)	0 (0)
with viable fetus	18 (100)	18 (100)	17 (94.4)	15 (100)
Total Females Gravid	18 (90)	20 (100)	18 (90)	16 (80)

[1]Adapted from original report, p. 21.
[2]Statistical evaluation not performed.

Gross Pathology

Maternal Necropsy Observations

Gross abnormalities were not noted in the treatment groups at necropsy.

Cesarean Section Observations

No treatment related differences were noted in the mean Cesarean section parameters evaluated between the control and 50 mg/kg/day groups (Table 5).

A decrease in the mean number of viable fetuses was noted at the 200 and 500 mg/kg/day dose levels compared to the control. At the high dose level, this decrease could be correlated with the mean number of statistically significant late resorptions at the high dose level (1.1 versus 0.2 in the control). The mean post-implantation loss percentage showed a dose related value of 7.3%, 8.7%, 11.7% and 21.9%, respectively, in the control, low, middle and high dose level. The high dose level mean value showed statistical significance ($p<0.05$) (Table 5).

The mean fetal sex ratio at the 50 mg/kg/day level showed a statistically significant ($p<0.05$) predominance of male fetuses (62.8%) versus the controls. Male fetuses also predominated at the higher dose levels (Table 5).

Table 5

Cesarean Section Observations[1]

	Dose (mg/kg/day)			
	0	50	200	500
Females Gravid:	18	18	18	15
Implantation Sites				
Total	138	138	120	105
per litter	7.7	7.7	6.7	7.0
Pre-implantation Loss				
Total	62	68	70	67
per litter	3.4	3.8	3.9	4.5
Viable Fetuses				
Total	128	126	106	82
per litter	7.1	7.0	5.9	5.5
Dead Fetuses				
Total	0	0	0	1
per litter	0	0	0	0.1
Late Resorptions				
Total	3	9	2	16
per litter	0.2	0.5	0.1	1.1
Early Resorptions				
Total	7	3	12	6
per litter	0.4	0.2	0.7	0.4
Post-implantation Loss				
Total	10	12	14	23
per litter	0.6	0.7	0.8	1.5[3]
%[2,3]	7.3	8.7	11.7	21.9
Sex Ratio (M/F)				
Total	62/66	79/47	58/48	47/35
per litter	3.4/3.7	4.4/2.6[3]	3.2/2.7	3.1/2.3
% Males[4]	47.9	62.8	54.2	57.4

[1]Adapted from original report, p. 33-34.
[2][# of implantations - # of viable fetuses/# implantations] x 100]; calculated by reviewer.
[3]Statistically significant ($p<0.05$).
[4]Calculated by reviewer.

Fetal Morphological Observations

The control and the treated groups showed a similar incidence of total dissimilar malformations (Table 6).

It was noted that the concurrent control values for total fetuses and litters showing hydrocephaly were slightly outside the historical control range. The malformation was seen in 3 fetuses from 1 litter in the control group, 3 fetuses in 2 litters from the

200 mg/kg/day group and 10 fetuses from 3 litters from the 500 mg/kg/day group (Table 6). With the exception of 1 fetus in the 200 mg/kg/day dose group, all fetuses with hydrocephaly were noted to have a domed head.

In the fetuses, an increased incidence of hydrocephaly ($p<0.05$) was noted between the control and the treated group at the 500 mg/kg/day dose level. When the number of litters showing the malformation were compared to the control values, an increased incidence occurred at the 200 and 500 mg/kg/day. The "Exact Trend" test showed a statistical significant trend ($p=0.04$) although statistical significance was not seen at the individual dose levels (Table 6).

Table 6

Number of Fetal Malformations[1]

Dose Level (mg/kg/day)

	Fetuses				Litters			
	0	50	200	500	0	50	200	500
No. Examined Externally	128	126	106	82	18	18	17	15
Filamentous tail	0	0	1	0	0	0	1	0
Microphthalmia	0	0	0	1	0	0	0	1
Flexed paw	0	0	0	2	0	0	0	2
Spina bifida	0	1	0	0	0	1	0	0
No. Examined Viscerally	128	126	106	82	18	18	17	15
Iris bombe	2	1	0	1	2	1	0	1
Unascended kidney	0	0	1	0	0	0	1	0
Lung cysts	0	1	0	0	0	1	0	0
Hydrocephaly[2,3]	3	0	3	10	1	0	2	3
%[4,5]	2.3	0	2.8	12.2	5.6	0	11.8	20
No. Examined Skeletally	128	126	106	82	18	18	17	15
Vertebral Anomaly with/or without rib anomaly	0	0	1	0	0	0	1	0
Skull Anomaly	1	0	1	1	1	0	1	1
Rib Anomaly	0	0	0	1	0	0	0	1
Total Malformations								
External	0	1	1	3	0	1	1	2
Soft tissue	5	2	4	11	3	2	3	4
Skeletal	1	0	2	2	1	0	2	2

[1]Adapted from original report, p. 35.
[2]Statistical significance ($p < 0.05$).
[3]Value recalculated from the original report.
[4]Fetus historical control data from original report p. 222. Range 0 - 1.3%.
[5]Litter historical control data from original report p. 222. Range 0 - 5.3%.

Discussion:

A minimal reduction in the body weight gain and a slight weight loss occurred at the 500 mg/kg/day dose levels during gestation days 12-19 and 15-19, respectively. At the 200 mg/kg/day dose level, minimal body weight loss occurred during gestation days 15-19. The weight changes during gestation days 15-19 could be correlated with a slight decrease in food consumption at the same time period. These slight changes in the rate of weight gain and food consumption were not statistically significant and did not demonstrate maternal toxicity (Table 2,3).

Even though no statistical significance was noted in the weight change/decreased body weight and decrease food consumption, the authors considered the above changes a sign of maternal toxicity. The reviewer, however, disagrees with this conclusion and considers the changes to be toxicologically not important.

The mean post-implantation loss percentage showed a dose related increase of 7.3%, 8.7%, 11.7% and 21.9%, respectively, in the control, low, middle and high dose level which correlated with a decrease in the mean number of viable fetuses at the 200 and 500 mg/kg/day dose levels. At the middle dose level, the decrease was caused by an increase in the early resorptions, not seen in the high dose level animals. This effect was toxicologically not important (Table 5).

According to the Springborn Laboratories historical control data for New Zealand White rabbits (p. 221 of the original report), the percent males versus females fetuses was 48.0% which was similar to the control percent of 47.9%. However, the mean fetal sex ratio at the 50 mg/kg/day level showed a predominance of male fetuses (62.8%) versus the controls ($p<0.05$). A similar although not statistically significant difference was also seen in the 200 (54.2% males) and 500 (57.4% males) mg/kg/day levels (Table 5). This sex ratio change was not considered related to treatment because of the lack of statistical significance and because the sex ratio at the both the 200 and 500 mg/kg/day levels were of a similar magnitude and a dose response was not present.

The number of fetuses showing hydrocephaly was 3/128 (2.3%) in the control group, 0/126 (0%) in the low dose, 3/106 (2.8%) in the mid dose, and a statistical significant 10/82 (12.2%) in the high dose level. The number of litters with fetuses exhibiting hydrocephaly was 1/18 (5.6%) in the control, 0/18 (0%) in the low dose, 2/17, (11.8%) in the mid dose and 3/15 (20.0%) in the high dose level. (Table 6).

Comparison of the fetuses data to the respective control and the historical control data lead to the conclusion that the 500 mg/kg/day dose level showed evidence of a treatment-related increase in the fetal incidence of hydrocephaly. When the litter

data were analyzed using the Exact Trend test, a statistically significant difference (p=0.04) was seen, although statistical significance was not seen in pairwise comparisons.

Comparison of the study data to the historical controls show that hydrocephaly was noted in 3/1957 fetuses (0.0-1.3%). Two litters per 274 showed this defect (0.0-5.3%). Both the fetus and the litter values in this study were higher than the historical controls at all dose levels. The reason for this difference is not know. More weight, therefore, was given to the concurrent controls for data evaluation.

Conclusions:

Monosodium Isocyanurate was administered to pregnant New Zealand White rabbits as a single daily dose by gavage at dose levels of 0 (Control), 50, 200 and 500 mg/kg/day from day 6 to 19 of gestation.

Maternal toxicity was not observed during the study.

A decrease in the mean number of viable fetuses was noted at the 500 mg/kg/day dose level when compared to the control group. This was caused by an increase in late resorptions and reflected in the mean post-implantation loss incidence of 21.9% versus the control incidence of 7.3%.

Comparison of the fetal data to their respective controls indicated that the 500 mg/kg/day dose had a 12.2% incidence of hydrocephaly versus 2.3% in the controls. The incidence of hydrocephaly showed a dose related trend when the litter data was analyzed.

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