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Developmental Toxicity of Cysteamine in the Rat: Effects on Embryo–Fetal Development

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ABSTRACT The reproductive and developmental safety of cysteamine has become an important issue to children with cystinosis because renal transplants and treatment with cysteamine reduce the complications associated with cystinosis and increase the lifespan of the affected children. In addition, there is the potential to decrease the severity or the incidence of renal Fanconi syndrome with administration of cysteamine to pregnant women carrying fetuses with cystinosis, and to ease significantly the burden of this disease throughout their lives. If cysteamine increases significantly the risk of fetal death, growth retardation or birth defects at doses used to treat women with cystinosis, treatment of the affected female should cease during pregnancy and would not be considered for fetal treatment. The goal of this study was to assess the developmental safety of exposure in utero to cysteamine in the rat. Pregnant rats were given cysteamine (as phosphocysteamine) from day 6.5 through day 18.5 postconception and fetuses were assessed for survival, growth, and structural abnormalities on day 20.5. Cysteamine was administered orally in doses of 0, 37.5, 75, 100, or 150 mg/kg/day. Cysteamine produced dose-dependent developmental toxicity with an apparent no adverse effect observed level of 75 mg/kg/day. Specific malformations were associated with this effect (cleft palate, kyphosis), as well as intrauterine growth retardation and fetal death at 100–150 mg/kg/day, without signs of maternal toxicity. Investigations continue into the mechanism for the developmental toxicity of cysteamine. *Teratology 58: 96–102, 1998.* · 1998 Wiley-Liss, Inc.

Cystinosis, first described in 1903 (Abderhalden, '03), is an autosomal recessive metabolic disease in which the amino acid cystine accumulates in lysosomes due to a defect in lysosomal cystine transport and leads to cellular death (Gahl, '86; Gahl et al., '88). The incidence is estimated to be between 1 and 2 in 200,000 live births (Gahl, '86). Cystinosis in infancy is associated with renal Fanconi syndrome (impairment of proximal renal tubular resorption), poor growth, muscle wastage, and renal failure. Renal transplantation enables children with cystinosis to survive the inevitable renal failure, but the children receiving kidney transplants suffer from complications accompanying the degeneration of other body tissues (Fink et al., '89; Schneider et al., '90; Theodoropoulos et al., '95; Charnas et al., '94). Cysteamine (NH_2—CH_2—CH_2—SH, β-mercaptoethylamine) prevents the excess storage of cystine in lysosomes (Thoene et al., '76; Butler and Zatz, '84).

The reproductive and developmental safety of cysteamine has become an important issue to children with cystinosis because renal transplants and treatment with cysteamine reduce the complications associated with cystinosis. In addition, there is the potential to decrease the severity or the incidence of renal Fanconi syndrome with administration of cysteamine to pregnant women carrying fetuses with cystinosis, and ease significantly the burden of this disease throughout their lives (Gahl, '86). If cysteamine increases significantly the risk of fetal death, growth retardation, or birth defects at doses experienced by cystinotic patients, treatment of the affected female would cease during pregnancy and would not be considered for fetal treatment.

The literature concerning the reproductive and developmental toxicity of cysteamine is meager. Literature searches identified only four full publications which report fetal outcome after in utero exposure to potentially toxic doses of cysteamine in laboratory animals: Adams et al. ('61), Reyss-Brion ('62), Beliles and Scott ('67), and Manowska and Mazur ('88). Only two of these reports involve exposures which included the period of organogenesis, namely Reyss-Brion ('62) and Beliles and Scott ('67).

Reyss-Brion ('62) reported that cysteamine reduced the percent of embryos surviving, and increased the incidence of malformed embryos in the chick. While this study indicates a potential for general toxicity, exposures in mammalian species generally yield data more relevant to evaluating human safety issues.

Beliles and Scott ('67) exposed male and female rats to cysteamine in their food for 70 days before mating

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and then continued the maternal exposure throughout gestation and lactation. Reduced litter size was observed in the group receiving the highest exposure, 375 mg/kg/day. An increase in malformations was not seen but, because natural delivery was permitted and dams may have eaten severely affected offspring, the data may not accurately represent the actual occurrence of abnormalities. Postnatal growth and survival were reduced when the dam continued to consume cysteamine at an exposure of 375 mg/kg/day. The mechanism for the postnatal effects is not known.

We have shown previously (Assadi et al., '98) that 150 mg/kg/day cysteamine produced a nonsignificant decrease in body weight gain during pregnancy to day 6.5 postconception, a significant increase in liver and spleen weights, and a significant increase in days to coitus; but there were no adverse effects on conception and early embryonic development.

In this study, we report a maternal dose-fetal outcome relationship for the developmental toxicity of cysteamine after oral administration of different doses of cysteamine, as phosphocysteamine (Medea Research Laboratories, Port Jefferson Station, NY), from day 6.5 through day 18.5 postconception. The doses of cysteamine are the same as were used in our previous study of the effects of cysteamine on female reproduction and early embryonic development (Assadi et al., '98). Hydrolysis of phosphocysteamine in the gastrointestinal tract rapidly produces equimolar quantities of cysteamine; thus, administration of phosphocysteamine is equivalent to giving cysteamine minus the objectionable taste of cysteamine (Schneider et al., '95).

MATERIALS AND METHODS

Virgin female rats (Wistar strain, Charles River Breeding Laboratories, Wilmington, MA) weighing approximately 200 gm were acclimated to the animal facility for 1 week in individual cages. Food and water were provided ad libitum. The dams were then placed overnight, 4 p.m. until 8 a.m., in cages with male rats of the same strain. Sperm found in the vaginal lavage was used to define 0.5 days postconception. Pregnant rats were housed individually. Each dam was weighed daily, observed for signs of ill health, and food consumption was determined daily.

On day 6.5 through day 18.5 postconception, cysteamine was administered between 8 and 9 a.m. by oral injection using a curved feeding tube with a ball tip. The daily doses were 0, 37.5, 75, 100, or 150 mg of cysteamine per kg body weight. The cysteamine was administered in a 10% (w/v) aqueous solution; the control (0 mg/kg) group received the volume of water equivalent to that in the highest dose group. There were 20 dams in each group.

On day 20.5 postconception, the dams were weighed and euthanized by cervical dislocation after induction of CO_2 narcosis. Each uterus was opened along its length and examined for signs of intrauterine death. After removing fetuses and placentae, each uterus with attached ovaries was immersed in 10% ammonium sulfide for 10 min to permit identification of additional sites not readily visible otherwise and to improve visualization of corpora lutea.

The surviving fetuses and their placentae were weighed separately. External fetal morphology was examined for gross abnormalities. The fetuses were euthanized with an oral administration of 20–40 µl Sleepaway (Fort Dodge Laboratories, Fort Dodge, IA) and a fresh visceral examination for abnormalities was conducted using the method of Staples ('74) as modified by Stuckhardt and Poppe ('84). After completion of the visceral examination, the head was removed from each even-numbered fetus and placed in Bouin's fixative. After a minimum of 2 weeks in this solution, the fixative was replaced by 70% ethanol. The heads were free-hand sectioned, using the method of Wilson ('65), to permit examination of the brain and eyes. The intact fetuses and the bodies of those from which the heads were removed were prepared for skeletal examination using a double-staining procedure of Kimmel and Trammell ('81) as modified by Webb and Byrd ('94), in which alcian blue was used to stain cartilage, and alizarin red S was used to stain bone.

Statistical analyses

All dependent variables were on the interval level of measurement. Dependent variables measured on multiple occasions were analyzed using repeated measures Analysis of Variance (ANOVAs) (e.g., daily food consumption and daily body weight). Significant findings were followed up by dependent *t*-tests corrected for multiple contrasts by the Bonferroni adjustment (main effects for time) or one-way ANOVAs, with the post hoc, experimentwise error control through the Tukey adjustment.

Dependent variables measured on one occasion were analyzed using one-way ANOVAs, with post hoc comparisons completed through the Tukey correction. The level of significance was $P < .05$, values are mean ± SD.

RESULTS

Maternal body weight and food consumption

The animals administered 150 mg cysteamine per kg per day experienced a decreased weight gain during pregnancy on days 10.5 through 30.5 (Table 1). Statistical analysis of the values on day 20.5 revealed a significant group × time interaction ($F = 9{,}513.23$, df[4,4], $P < .001$). Post hoc analysis showed a significant difference ($P < .05$) between the 150 mg/kg/day group and all the other groups.

Food consumption (Table 2) decreased on day 7.5 in all groups receiving cysteamine. On day 8.5, food consumption was decreased in the 100 and 150 mg/kg/day groups. Decreased food consumption by rats in the 150 mg/kg/day group continued until day 13.5.

Statistical analysis of the values for food consumption on day 20.5 revealed a significant main effect for

TABLE 1. Body weight of pregnant rats administered different doses of cysteamine from day 6.5 through 18.5 postconception*

Day of pregnancy	Cysteamine administered, mg/kg/day				
	0	37.5	75	100	150
Dams	17	19	17	18	15
0.5	210 ± 25	202 ± 14	207 ± 14	210 ± 10	208 ± 8
1.5	213 ± 22	204 ± 14	210 ± 14	212 ± 12	209 ± 8
2.5	217 ± 22	208 ± 13	217 ± 14	216 ± 11	212 ± 11
3.5	222 ± 16	214 ± 13	222 ± 12	221 ± 12	218 ± 9
4.5	228 ± 22	220 ± 12	227 ± 12	226 ± 12	224 ± 9
5.5	234 ± 21	226 ± 13	231 ± 15	231 ± 12	229 ± 9
6.5	237 ± 21	228 ± 12	235 ± 12	234 ± 12	231 ± 10
7.5	241 ± 20	228 ± 13	230 ± 14	230 ± 14	225 ± 12
8.5	246 ± 18	232 ± 13	237 ± 11	234 ± 12	228 ± 15
9.5	252 ± 20	237 ± 15	241 ± 15	238 ± 12	228 ± 15
10.5	257 ± 18	243 ± 14	246 ± 14	243 ± 12	231 ± 16**
11.5	263 ± 20	248 ± 15	251 ± 16	249 ± 10	233 ± 16**
12.5	267 ± 19	254 ± 15	255 ± 15	253 ± 11	234 ± 14**
13.5	273 ± 20	258 ± 17	262 ± 14	261 ± 13	236 ± 15**
14.5	279 ± 21	266 ± 17	267 ± 15	264 ± 14	238 ± 19**
15.5	288 ± 20	273 ± 17	272 ± 19	274 ± 19	242 ± 20**
16.5	299 ± 21	284 ± 16	282 ± 21	278 ± 16	247 ± 24**
17.5	310 ± 20	297 ± 18	294 ± 25	290 ± 15	252 ± 29**
18.5	326 ± 25	313 ± 21	307 ± 24	304 ± 18	256 ± 31**
19.5	343 ± 23	320 ± 31	315 ± 30	319 ± 19	261 ± 34**
20.5	361 ± 25	341 ± 25	332 ± 30	334 ± 21	265 ± 39**

*Values are mean ± SD.
**Significantly different from the value for the 0 mg/kg/day group, $P < .05$.

TABLE 2. Food consumption by pregnant rats administered different doses of cysteamine from day 6.5 through 18.5 postconception*

Day of pregnancy	Cysteamine administered, mg/kg/day				
	0	37.5	75	100	150
Dams	17	19	17	18	15
1.5	22 ± 3	20 ± 3	21 ± 4	22 ± 5	20 ± 4
2.5	25 ± 4	24 ± 4	25 ± 3	24 ± 3	23 ± 6
3.5	27 ± 4	26 ± 4	26 ± 3	25 ± 5	24 ± 3
4.5	28 ± 4	27 ± 2	27 ± 5	28 ± 7	26 ± 4
5.5	28 ± 4	27 ± 2	27 ± 5	27 ± 4	26 ± 3
6.5	28 ± 3	27 ± 4	28 ± 3	26 ± 3	26 ± 6
7.5	28 ± 3	22 ± 4**	18 ± 5**	19 ± 6**	15 ± 5**
8.5	28 ± 3	24 ± 4	24 ± 5	22 ± 4**	20 ± 7**
9.5	28 ± 2	25 ± 4	26 ± 5	24 ± 3	16 ± 5**
10.5	28 ± 3	26 ± 3	26 ± 5	26 ± 5	19 ± 6**
11.5	28 ± 3	26 ± 2	27 ± 5	26 ± 4	21 ± 7**
12.5	28 ± 4	29 ± 3	28 ± 5	28 ± 4	21 ± 8**
13.5	29 ± 4	29 ± 4	30 ± 6	28 ± 4	21 ± 7**
14.5	29 ± 4	29 ± 3	29 ± 3	28 ± 6	24 ± 8
15.5	29 ± 3	29 ± 4	27 ± 7	27 ± 4	26 ± 7
16.5	32 ± 4	30 ± 4	30 ± 6	30 ± 6	27 ± 7
17.5	31 ± 5	31 ± 4	30 ± 5	29 ± 5	27 ± 8
18.5	32 ± 7	33 ± 4	32 ± 5	32 ± 6	26 ± 6
19.5	32 ± 4	32 ± 3	29 ± 6	31 ± 4	25 ± 4
20.5	32 ± 4	31 ± 3	33 ± 3	33 ± 4	29 ± 6

*Values are mean ± SD food consumption during the previous 24 hr.
**Significantly different from the value for the 0 mg/kg/day group, $P < .05$.

time ($F = 80.97$, df[4,1], $P < .001$). No dose-associated difference was detected ($F = 2.31$, df[4,4], $P = .065$) nor was the group × time comparison significant ($F = 1.24$, df[4,4], $P = .302$). Thus, food consumption was decreased for 7 days in the 150 mg/kg/day group but was not statistically different on the other groups during the last 7 days, until sacrifice on day 21.5. Clinical signs of toxicity were not observed in the dams.

Reproductive and fetal outcome

Tables 3 and 4 summarize the reproductive and fetal outcome of this experiment. The number of corpora

TABLE 3. Reproductive outcome after maternal exposure to different doses of cysteamine from day 6.5 through day 18.5 postconception*

Reproductive outcome	Cysteamine administered, mg/kg/day				
	0	37.5	75	100	150
Pregnant dams	17	19	17	18	15
Corpora lutea/pregnant dam	16.8 ± 2.0	16.3 ± 3.0	15.9 ± 2.6	16.2 ± 2.0	15.4 ± 1.6
Implantation sites/litter	14.4 ± 1.6	13.6 ± 2.5	12.9 ± 3.2	13.7 ± 1.6	12.7 ± 2.1

*Pregnant rats were sacrificed on day 20.5. Values are mean ± SD.

TABLE 4. Fetal outcome after maternal exposure to different doses of cysteamine from day 6.5 through day 18.5 postconception*

Fetal outcome	Cysteamine administered, mg/kg/day				
	0	37.5	75	100	150
Live fetuses, total	234	246	188	210	35
Live fetuses/litter	13.8 ± 1.8	12.9 ± 2.7	11.1 ± 3.7	11.7 ± 1.9	2.3 ± 3.2**
Fetal body weight/litter, gm	3.67 ± 0.22	3.51 ± 0.24	3.38 ± 0.27	3.15 ± 0.23**	2.58 ± 0.36**
Male body weight/litter, gm	3.75 ± 0.25	3.53 ± 0.30	3.45 ± 0.24	3.19 ± 0.26**	2.66 ± 0.18**
Female body weight/litter, gm	3.58 ± 0.23	3.52 ± 0.28	3.27 ± 0.38	3.15 ± 0.33**	2.54 ± 0.21**
Placental weight/litter, gm	0.48 ± 0.05	0.48 ± 0.04	0.51 ± 0.07	0.46 ± 0.05	0.43 ± 0.06***
Abnormal fetuses/litter (%/litter)	0.1 ± 0.3 (1%)	0.2 ± 0.4 (2%)	0.4 ± 1.1 (3%)	4.5 ± 4.8 (41%)**	1.7 ± 2.6 (88%)**
Fetuses with IUGR/litter (%/litter)	0.4 ± 0.9 (3%)	0.8 ± 1.3 (6%)	0.5 ± 0.9 (4%)	3.4 ± 3.6 (31%)**	2.1 ± 3.1 (86%)**
Male fetuses with IUGR/litter	0.2 ± 0.4	0.5 ± 1.3	0.1 ± 0.2	1.1 ± 1.3**	1.1 ± 1.6**
Female fetuses with IUGR/litter	0.1 ± 0.2	0.2 ± 0.4	0.2 ± 0.4	1.4 ± 1.9**	1.0 ± 1.7**
Intrauterine deaths/litter (%/litter)	0.5 ± 0.7 (4%)	0.7 ± 1.1 (5%)	1.8 ± 2.3 (15%)	2.0 ± 1.7 (14%)	10.4 ± 3.0 (83%)**

*Values are mean ± SD. Pregnant rats were sacrificed on day 20.5. IUGR (intrauterine growth retardation) was arbitrarily defined as fetal body weight 3 SD below that of the mean in the 0 mg/kg/day group (i.e., <3.00 gm for male and <2.88 gm for female fetuses).
**Significantly different from the value for the 0 mg/kg/day group, $P < .001$.
***Significantly different from the value for the 0 mg/kg/day group, $P < .01$.

lutea and the number of implantations sites were similar ($P > .05$) in all groups (Table 2).

Measures of adverse fetal outcome included intrauterine death, intrauterine growth retardation, abnormalities of external and visceral structure, and abnormalities of skeletal development. Taken together, adverse fetal outcome was significantly increased in the 100 and 150 mg/kg/day groups ($F = 50.9161$, df[4,81], $P < .001$) (Table 4). Tables 5 and 6 summarize the fetal and litter incidence of the abnormalities and variations observed.

Intrauterine death

The number of live fetuses was significantly decreased ($F = 44.224$, df[4.81], $P < .001$) in the 150 mg/kg/day group only and the number of intrauterine deaths was significantly increased in this same group ($F = 74.37$, df[4,81], $P < .001$) (Table 4). Fetal death occurred on about day 11 of pregnancy or later, as judged by the size of the remaining placenta or the developmental stage of the fetal remains (Christie, '64).

Intrauterine growth retardation

The average fetal body weight was significantly decreased in groups exposed to 100 and 150 mg/kg/day ($F = 29.9$, df[4,73], $P < .001$) (Table 4). The average placental weight was significantly decreased only in the 150 mg/kg/day group ($F = 3.79$, df[4,74], $P < .01$). Intrauterine growth retardation (IUGR) in male and female fetuses was arbitrarily defined as a fetal body weight greater than 3 SD below the mean body weights for male and female fetuses measured in the 0 mg/kg/day group. Using this criterion, the numbers of male and female fetuses with intrauterine growth retardation were similarly and significantly increased ($P < .001$) in the 100 and 150 mg/kg/day groups.

External and visceral development

The number of live fetuses with malformations was significantly increased ($P < .001$) in the 100 and 150 mg/kg/day groups (Table 4). The most common morphological abnormalities associated with exposure to cysteamine were cleft palate (without cleft lip) and kyphosis (Table 5).

Skeletal development

Analysis of fetal skeletons after staining with alizarin red and alcian blue (Tables 5 and 6) revealed an increase in the incidence of kyphosis and abnormalities of the vertebrae associated with exposure to cyste-

TABLE 5. Incidence of abnormalities and variations in fetuses (excluding eyes, brain, and skeleton of the head) after maternal exposure to different doses of cysteamine from day 6.5 through day 18.5 postconception*

Abnormalities and variations	Cysteamine administered, mg/kg/day				
	0	37.5	75	100	150
Litters examined	*17*	*19*	*17*	*18*	*15*
Fetuses examined	*234*	*246*	*188*	*210*	*35*
Head					
Ears, low set	—	1/1	—	—	1/1
Palate, cleft	—	—	2/2	34/6	24/6
Axis (vertebrae)					
Kyphosis	1/1	2/1	9/3	79/14	31/7
Scoliosis	—	—	—	1/1	—
Vertebrae, asymmetric	—	—	2/1	1/1	—
Vertebrae, agenesis of entire regions	—	2/2	—	—	1/1
Vertebrae, rachishisis	—	1/1	1/1	1/1	3/1
Centrum, bipartite	2/2	1/1	3/2	3/2	—
Sternum and ribs					
Sternum, bifid	—	3/3	—	—	—
Sternebrae, asymmetric	—	—	—	—	1/1
Sternebrae, checkerboard ossification	—	—	—	1/1	—
Ribs, wavy	31/10	19/5	18/8	67/13	19/6
Ribs, bulbous or nodes	12/8	9/4	5/4	20/5	11/4
Ribs, thickened	22/8	7/4	14/6	64/13	15/6
Ribs, supernumerary	10/5	2/2	9/3	2/2	—
Ribs, missing	—	2/2	—	—	—
Thoracic region					
Heart, ventricular septal defect	—	—	1/1	2/2	—
Kidney, necrotic, unilateral	—	—	—	1/1	—
Pelvic region					
Kink in tail	—	1/1	—	—	—
Arthrogryposis plantar contraction	—	—	4/2	2/2	4/4

*Non-italic values are fetuses affected/litters affected. Pregnant rats were sacrificed on day 20.5.

amine. An increase in variations of rib morphology was also noted, including wavy rib, nodes, and thickening of ribs. The significance of this finding is not known because variations of rib morphology were common in the 0 mg/kg/day group.

DISCUSSION

Our results show that cysteamine produced dose-dependent adverse effects on the fetus during organogenesis and histogenesis. Specific malformations can be associated with this effect (cleft palate, kyphosis, vertebral anomalies), as well as IUGR and fetal death. Neither 37.5 nor 75 mg/kg/day produced a significant increase in adverse effects on the fetus. While adverse effects were seen in the 100 mg/kg/day group, the most severe effects on fetal outcome were seen in the 150 mg/kg/day group, in which there was no apparent maternal toxicity. A daily administration of 75 mg/kg is within the therapeutic dose (60 to 90 mg/kg/day), but patients are given this dose in four equal doses. Unfortunately, a pharmacokinetic evaluation of cysteamine in the mother and fetus after an oral dose of phosphocysteamine has not been determined, to our knowledge, in the rat.

The animals receiving the greatest dose of cysteamine, 150 mg/kg/day, experienced a decrease in weight gain during pregnancy. The reduction in pregnancy weight gain can be partly explained by a period of reduced food consumption. From Table 2, the food consumption by the dams treated with 150 mg/kg/day can be calculated to be about 53% of the value for the 0 mg/kg/day group on day 7.5 then increasing to 75% by day 13.5. The values on day 14.5 through day 20.5 are not statistically different from those of the 0 mg/kg/day group. During this period, there was significant fetal death in the 150 mg/kg/day group (Table 4). The fetal loss must also have contributed to the decreased weight gain of the dams.

In both rats and humans, the effects of cysteamine are mediated by four generally accepted mechanisms; namely, antioxidant (Bacq, '65; Huxtable, '92), interactions between the thiol of cysteamine with proteins, depletion of somatostatin (Vecsei and Widerlov, '90), and inhibition of the glycine cleavage system (Yudkoff et al., '81). At this time, it is not known whether these mechanisms are involved in the adverse fetal effects observed in this study.

TABLE 6. Incidence of abnormalities in eyes, brain and skeleton of the head of fetuses after maternal exposure to different doses of cysteamine from day 6.5 through day 18.5 postconception*

	Cysteamine administered, mg/kg/day				
Abnormalities	0	37.5	75	100	150
Litters examined	*17*	*19*	*17*	*18*	*15*
Fetal heads examined for eye and brain anomalies	*113*	*116*	*89*	*94*	*15*
Brain, microcephaly	—	—	—	—	1/1
Retina, displaced, bilateral	—	—	—	1/1	—
Fetal heads examined for skeletal anomalies	*121*	*130*	*99*	*116*	*20*
Mandible, short	—	—	—	8/2	3/3
Mandible, angulated	—	—	—	13/4	1/1
Mandible, asymmetric	1/1	—	—	—	—
Naris/nasal misshapen	—	12/4	15/6	33/9	15/7
Maxilla misshapen	1/1	1/1	—	—	—
Fused parietal, temporal, and frontal bones	—	1/1	1/1	—	—
Fused parietal, temporal, and interparietal bones	5/3	5/4	5/3	5/1	4/3
Supraoccipital bone, aplasia	—	—	—	1/1	—
Hyoid, misshapen	2/1	2/2	—	2/2	1/1

*Non-italic values are fetuses affected/litters affected. Pregnant rats were sacrificed on day 20.5.

An evaluation of the effects of cysteamine on early development in the rat (Assadi et al., '98) revealed no adverse effects on conception and early embryonic development. In that study, the results suggested that a low level of toxicity was manifested in the dams exposed to 150 mg/kg/day for 5 to 7 weeks.

The results reported herein demonstrate a dose-response relationship for the developmental toxicity of cysteamine. Adverse fetal outcome included malformations, intrauterine growth retardation, and fetal death at doses which did not produce clinical signs of maternal toxicity. Although no statistically significant increase in adverse effects were seen at 75 mg/kg/day, a full interpretation of this apparent no observed adverse effect level must await the determination of the pharmacokinetics of cysteamine in the pregnant rat.

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LITERATURE CITED

Abderhalden, E. (1903) Familiare cystindiathese. Z. Physiol. Chem., *38*:557.

Adams, C.E., M.F. Hay, and C. Lutwak-Mann (1961) The action of various agents upon the rabbit embryo. J. Embryol. Exp. Morph., *9*:468–491.

Assadi, F.K., J.J. Mullin, and D.A. Beckman (1998) Evaluation of the reproductive and developmental safety of cysteamine in the rat: Effects on female reproduction and early embryonic development. Teratology, *58*:88–95.

Bacq, Z.M. (1965) Metabolism and distribution in mammals. In: Chemical Protection Against Ionizing Radiation. Z.M. Bacq, ed. Springfield, IL: Thomas, pp. 96–115.

Beliles, R.P., and W.J. Scott, Jr. (1967) Effect of beta-mercaptoethylamine on reproduction of the rat. Toxicol. Appl. Pharmacol., *11*:523–528.

Butler, J.D., and M. Zatz (1984) Pantethine and cysteamine deplete cystine from cystinotic fibroblasts via efflux of cysteamine-cystine mixed disulfide. J. Clin. Invest. *74*:411–416, 1984.

Charnas, L.R., C.A. Luciano, M. Dalakas, R.W. Gilliatt, I. Bernardini, K. Ishak, V.A. Cwik, D. Fraker, T.A. Brushart, and W.A. Gahl (1994) Distal vacuolar myopathy in nephropathic cystinosis. Ann. Neurol., *35*:181–188.

Christie, G.A. (1964) Developmental stages in somite and post-somite rat embryos, based on external appearance, and including some features of the macroscopic development of the oral cavity. J. Morph. *114*:263–286.

Fink, J.K., P. Brouwers, N. Barton, M.H. Malekzadeh, S. Sato, S. Hill, W.E. Cohen, B. Fivush, and W.A. Gahl (1989) Neurologic complications in long-standing nephropathic cystinosis. Arch. Neurol., *46*:543–548.

Gahl, W.A. (1986) Cystinosis. Coming of age. Adv. Pediatr., *33*:95–126.

Gahl, W.A., J.G. Thoene, J.A. Schneider, S. O'Regan, M.I. Kaiser-Kupfer, and T. Kuwabara (1988) NIH conference. Cystinosis: Progress in a prototypic disease. Ann. Internal Med., *109*:557–569.

Huxtable, R.J. (1992) Physiological actions of taurine. Physiol. Rev., *72*:101–163.

Kimmel, C.A., and C. Trammell (1981) A rapid procedure for routine double staining of cartilage and bone in fetal and adult animals. Stain Technol., *56*:271–273.

Manowska, J., and L. Mazur (1988) Quantity of glycogen in the liver of 19-day-old foetuses after AET, 5-HT, MEA, of GSH treatment of pregnant mice on the first day of gestation. Acta Physiol. Hung., *71*:51–54.

Reyss-Brion, M. (1962) Protection par la cysteamine de junes embryons de poulet soumis ulterieurement a une irradiation aux rayons x. Arch. Anat. Histol. Embryol. Norm. Exptl., *44*(Suppl.):197.

Schneider, J.A., K.F. Clark, A.A. Greene, J.S. Reisch, T.C. Markello, W.A. Gahl, J.G. Thoene, P.K. Noonan, and K.A. Berry (1995) Recent advances in the treatment of cystinosis. J. Inher. Metab. Dis. *18*:387–397.

Schneider, J.A., B. Katz, and R.B. Melles (1990) Update on nephropathic cystinosis. Pediatr. Nephrol., *4*:645–653.

Staples, R.E. (1974) Detection of visceral alterations in mammalian fetuses. Teratology, *9:*A37–A38.

Stuckhardt, J.L., and S.M. Poppe (1984) Fresh visceral examination of rat and rabbit fetuses used in teratogenicity testing. Teratogen. Carcinogen. Mutagen., *4:*181–188.

Theodoropoulos, D.S., T.H. Shawker, C. Heinrichs, and W.A. Gahl (1995) Medullary nephrocalcinosis in nephropathic cystinosis. Pediatr. Nephrol., *9:*412–418.

Thoene, J.G., R.G. Oshima, J.C., Crawhall, D.L. Olson, and J.A. Schneider (1976) Cystinosis: Intracellular cystine depletion by aminothiols in vitro and in vivo. J. Clin. Invest., *58:*180–189.

Vecsei, L., and E. Widerlov (1990) Preclinical and clinical studies with cysteamine and pantethine related to the central nervous system. Prog. Neuro-Pyschopharmacol. Biol. Psychiat., *14:*835–862.

Webb, G.N., and R.A. Byrd (1994) Simultaneous differential staining of cartilage and bone in rodent fetuses: An alcian blue and alizarin red S procedure without glacial acetic acid. Biotech. Histochem., *69:*181–185.

Wilson, J.G. (1965) Embryological considerations in teratology. In: Handbook of Teratology: Principles and Techniques. J.G. Wilson, and J. Warkany, eds. Chicago: University of Chicago Press, pp. 251–261.

Yudkoff, M., I. Nissim, A. Schneider, and S. Segal (1981) Cysteamine inhibition of [^{15}N]-glycine turnover in cystinosis and of the glycine cleavage system in vitro. Metabolism, *30:*1096–1103.