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FUNDAMENTAL AND APPLIED TOXICOLOGY 7, 33–40 (1986)

1/1/86 ROD

Evaluation of the Teratogenic Potential of Three Aliphatic Nitriles in the Rat[1]

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Evaluation of the Teratogenic Potential of Three Aliphatic Nitriles in the Rat. JOHANNSEN, F. R., LEVINSKAS, G. J., BERTEAU, P. E., AND RODWELL, D. E. (1986). *Fundam. Appl. Toxicol.* 7, 33–40. Acetonitrile (ACN), adiponitrile (ADN), and propionitrile (PN), were evaluated for embryotoxic and teratogenic potential in rats. Mated Sprague–Dawley rats were administered one of the three nitriles by gavage on gestation Days 6–19, inclusive. Daily dosage levels (mg/kg body wt) were: ACN at 0, 125, 190, and 275; ADN at 0, 20, 40, and 80; PN at 0, 20, 40, and 80. There was evidence of maternal toxicity in each of the high dose groups treated with ACN, ADN, or PN. Some maternal effects also were seen with ADN at the middle dosage. Embryotoxic effects were observed at the highest dosage tested for ACN and middle and high dose levels for PN. Slight fetotoxicity was observed at the highest dosage for ADN. No teratogenic effects were observed at any dosage level with ACN, ADN, or PN. © 1986 Society of Toxicology.

Aliphatic nitriles are used widely as chemical intermediates in the plastics, resins, and textiles industries. Relatively little information has been reported on the long-term effects related to exposure to most members of this class of compounds; even less information exists concerning their effects on the development of the fetus *in utero*.

No adverse affects were reported on reproduction in the offspring from the first generation of rats exposed to 500 ppm adiponitrile (Svirbely and Floyd, 1964). By contrast, propionitrile and acrylonitrile administered by intraperitoneal injection to hamsters on Day 8 of gestation were reported to induce several fetal malformations including exencephaly, encephaloceles, and rib defects (Willhite *et al.*, 1981). When administered to hamsters under the same dosing regimen, succinonitrile but not tetramethylsuccinonitrile produced fetal terata, including exencephaly, encephalocele, and runting (Doherty *et al.*, 1983).

The present study was undertaken to evaluate the teratogenic effects of three aliphatic nitriles using the standard rat test model. For this investigation, two mononitriles, acetonitrile (ACN) and propionitrile (PN), and a dinitrile, adiponitrile (ADN), were selected. The empirical formula for each is: ACN = CH_3CN; PN = C_2H_5CN; and ADN = $CN(CH_2)_4CN$.

MATERIALS AND METHODS

The same chemicals were used in both preliminary range-finding studies as well as the teratologic studies: ACN (>94%), was purchased from Mallinckrodt, Inc. (St. Louis, Mo.); ADN (>98%) and PN (>90%) were supplied by Monsanto Company (St. Louis, Mo.). The nitriles were dissolved in distilled water (ACN, ADN) or corn oil (PN) and administered by gavage in single daily doses to mated rats on Days 6–19 of gestation. All doses were administered at a constant volume of 10 ml/kg/day. Separate groups of untreated control rats were used for each nitrile. They received the corresponding vehicle on a comparable regimen.

[1] A portion of this work was presented at the 21st Annual Meeting of the Society of Toxicology, Boston, Mass.; February 1982.

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Animals used for all studies were sexually mature, virgin female rats (Charles River, COBS, CD) obtained from Portage, Michigan. Except during mating with a laboratory stock of proven males, all females were housed individually in wire-mesh cages and maintained in a temperature-, humidity-, and light-controlled environment. Certified Rodent Chow (Purina Co., St. Louis, Mo.) and water were available at all times. Following cohabitation, the day of mating was confirmed, either by observation of a copulatory plug or vaginal smears, and was designated Day 0 of gestation.

Preliminary range-finding studies were conducted for dose selection of test materials. In each case six groups of five pregnant female rats were administered solutions containing one of the three aliphatic nitriles: ACN at 0, 200, 375, 750, 1500, or 3000 mg/kg/day; ADN at 0, 10, 25, 50, 100, or 200 mg/kg/day; or PN at 0, 5, 10, 20, 37.5, or 75 mg/kg/day.

The following dosages were selected for the main studies designed to evaluate the teratogenic potential of each nitrile: ACN at 0, 125, 190, and 275 mg/kg/day; ADN at 0, 30, 50, and 80 mg/kg/day and PN at 0, 20, 40, and 80 mg/kg/day. These were administered to groups of 25 mated females using the same solvents as for the range-finding studies. A separate control group of 25 mated females was used with each nitrile.

In all studies, treated animals were observed daily for mortality and overt changes in appearance or behavior. Individual body weights were recorded on gestation Days 0, 6, 9, 12, 16, and 20. In the range-finding studies all surviving dams were sacrificed and uterine examinations were performed on gestation Day 20. In the teratogenic evaluation studies, uteri were excised and weighed prior to removal of fetuses. The number and location of viable and nonviable fetuses, early and late resorptions, and number of total implants and corpora lutea were recorded. All fetuses were individually weighed and examined for external malformations and variations. Approximately one-half of the fetuses were randomly selected and placed in Bouin's fixative and examined for visceral anomalies (Wilson, 1965). The remaining fetuses were fixed by conventional means (Dawson, 1926) and examined for skeletal alterations.

The male to female fetal sex distribution and number of litters with malformations were compared using the χ^2 test criterion with Yates' correction for 2×2 contingency tables and/or Fisher's exact probability test as described by Siegel (1956) to judge significance of differences. The number of early and late resorptions and postimplantation losses were compared by the Mann–Whitney U-test as described by Siegel (1956) and Weil (1970) to judge the significance of differences.

The mean number of viable fetuses, total implantations, corpora lutea, and mean fetal body weights were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances, and the appropriate t test (for equal or unequal variances) as described by Steel and Torrie (1960) using Dunnett's (1964) multiple comparison tables to judge significance of differences.

Only data from the main studies were analyzed statistically. In all cases, the treatment groups were compared to the control group with the level of significance at $p > 0.05$.

RESULTS

Range-Finding Studies

Acetonitrile. Several deaths considered related to treatment occurred. All test animals succumbed at and above levels of 750 mg/kg ACN while three of five rats died in the 375-mg/kg test group. Severe mean body weight losses and an increase in the mean number of early resorptions, were observed at and above 375 mg/kg. No effects on maternal toxicity or fetal survival were evident up to 200 mg/kg/day.

Adiponitrile. One of five treated females died in the 100-mg/kg group, while all rats administered 200 mg/kg ADN succumbed. Moderate to severe reductions in mean maternal body weight and a corresponding increase in mean postimplantation loss were observed at doses of 100 mg/kg and above. No treatment-related effects were noted up to 50 mg/kg/day ADN.

Propionitrile. No deaths occurred in this experiment. Moderate to severe reductions in mean maternal body weight and some evidence of increased early and late resorptions were observed at 75 mg/kg PN. No maternal effects and no embryotoxicity were observed up to 37.5 mg/kg/day.

Teratologic Evaluations

Acetonitrile. Four animals administered 275 mg/kg/day ACN appeared emaciated toward the end of the study while two others in this test group died (Table 1). Appearance and behavior of animals given 125 and 190 mg/kg/day ACN were unremarkable. Reduced mean body weight gain relative to controls was evident only at 275 mg/kg (Table 2). In the 125- and 190-mg/kg/day groups there were no differences relative to controls in mean numbers of viable fetuses, postimplantation loss, total

TABLE 1

OBSERVATIONS OF BRED RATS ADMINISTERED SELECTED NITRILES

Product dose level (mg/kg)[a]	No. deaths/ no. dosed	Pregnancy (%)	Maternal body wt (g)		Overall mean wt gain (g)
			Day 6	Day 20[b]	
Acetonitrile					
0	0/25	76	290 ± 22[c]	324 ± 30	34
125	0/25	96	283 ± 18	321 ± 19	38
190	0/25	92	272 ± 25	313 ± 25	41
275	2/25	92	285 ± 27	313 ± 36	28
Adiponitrile					
0	0/25	92	266 ± 23	292 ± 20	26
30	0/25	88	268 ± 27	297 ± 28	29
50	1/25	92	261 ± 19	289 ± 23	28
80	2/25	96	263 ± 19	296 ± 20	33
Propionitrile					
0	0/25	96	284 ± 20	319 ± 26	35
20	0/25	88	284 ± 18	317 ± 31	33
40	0/25	88	295 ± 19	334 ± 26	39
80	1/25	84	289 ± 23	314 ± 32	25

[a] Administered on gestation days 6–15.
[b] Adjusted weight (female weight minus uterus and contents).
[c] Mean ± SD.

TABLE 2

MATERNAL AND FETAL OBSERVATIONS AT SACRIFICE OF RATS ADMINISTERED SELECTED NITRILES

Product dose level (mg/kg)[a]	Total implants[b]	Resorptions[b]	Postimplant loss[b]	Live fetuses[b]	Fetal	
					Sex ratio (M:F)	Body wt (g)
Acetonitrile						
0	13.5 ± 3[c]	0.1 ± 0.2	0.6 ± 0.8[c]	12.8 ± 3[c]	55:45	3.7 ± 0.3[c]
125	13.0 ± 4	0 ± 0	0.8 ± 1.3	12.2 ± 4	49:51	3.7 ± 0.6
190	13.9 ± 2	0 ± 0	0.7 ± 0.7	13.2 ± 2	46:54	3.6 ± 0.2
275	14.0 ± 3	0 ± 0	2.5 ± 3.8	11.5 ± 6	53:47	3.6 ± 0.6
Adiponitrile						
0	13.5 ± 3	0 ± 0.2	0.7 ± 1.1	12.7 ± 4	54:46	3.6 ± 0.2
30	15.0 ± 2	0 ± 0.2	1.5 ± 3.4	13.5 ± 4	54:46	3.4 ± 0.4
50	13.5 ± 4	0 ± 0	0.8 ± 0.9	12.8 ± 4	52:48	3.7 ± 1.0
80	14.4 ± 8	0.1 ± 0.2	0.9 ± 1.1	13.5 ± 3	47:53	3.4 ± 0.3[d]
Propionitrile						
0	14.3 ± 3	0 ± 0	0.7 ± 0.8	13.6 ± 3	47:53	3.5 ± 0.3
20	14.2 ± 3	0 ± 0	0.5 ± 0.8	13.7 ± 3	54:46	3.5 ± 0.2
40	15.5 ± 2	0 ± 0	1.0 ± 0.9	14.5 ± 2	50:50	3.4 ± 0.2
80	15.7 ± 1	0.1 ± 0.2	2.0 ± 2.6[d]	13.7 ± 3	50:50	3.0 ± 0.4[e]

[a] Administered on gestation Days 6–15.
[b] Per dam.
[c] Group mean ± SD.
[d] Significantly different from the control values, $p < 0.05$.
[e] Significantly different from the control values, $p < 0.01$.

implantations, fetal sex distribution, or mean fetal weight. An increase in postimplantation loss (due to early resorptions), concomitant with a decrease in the mean number of fetuses, was observed at 275 mg/kg/day (Table 2). Skeletal and visceral observations did not result in statistically significant differences in the incidence of abnormalities when the groups were compared with one another (Table 3). Although within the historical control ranges at this laboratory (4–23%), the slightly increased incidence (23%) of unossified sternebrae (5 and 6) in the 275-mg/kg test group might be indicative of some embryotoxicity at this level.

Adiponitrile. One rat treated with 50 mg/kg/day ADN, and two dosed with 80 mg/kg/day, died (Table 1). Relative to their controls, there were no differences in appearance or behavior in any of the surviving animals in any

TABLE 3

INCIDENCE OF FETAL ALTERATIONS IN THE RAT FOLLOWING ACETONITRILE EXPOSURE

	Acetonitrile (mg/kg/day)[a]			
	0	125	190	275
	No. fetuses examined/No. litters examined			
External exam	244/19	293/24	303/23	242/19
Visceral exam	123/19	147/24	150/23	121/19
Skeletal exam	121/19	146/24	153/23	121/19
	No fetuses (No. litters) affected			
External exam				
Head anomaly	0	0	1 (1)	0
Dwarfism	0	3 (1)	0	0
Carpal flexure	1 (1)	0	0	0
Soft tissue exam				
Malformations				
Diaphragmatic hernia	0	0	1 (1)	0
Retroureter uterine horn	1 (1)	0	0	0
Developmental/genetic				
Renal papilla not developed	0	3 (3)	1 (1)	2 (2)
Major vessel variation	0	0	0	1 (1)
Skeletal exam				
Malformations				
Scoliosis and rib anomalies	0	0	0	1 (1)
Vertebral anomalies	0	0	1 (1)	0
Bent ribs	0	0	2 (2)	0
Developmental/genetic				
Vertebrae, extra 27 Presacral	0	0	0	2 (2)
Vertebrae, delayed ossification	0	0	0	1 (1)
Rib, 14th rudimentary	19 (9)	18 (12)	15 (9)	25 (12)
Hyoid, unossified	1 (1)	5 (5)	1 (1)	2 (2)
Skull, delayed ossification	0	4 (2)	4 (3)	2 (2)
Sternebrae, unossified (5th and 6th)	7 (4)	17 (12)	20 (9)	28 (11)

[a] Administered on gestation Days 6–15. No significant differences from control value were observed.

group. Maternal adjusted mean body weight gains were not affected at any dose level compared to the controls. Uterine and fetal examinations did not reveal any differences between treated and control groups in parameters measured at sacrifice (Table 2). There was a statistically significant ($p < 0.05$) decrease in mean fetal body weight at 80 mg/kg, but not 30 mg/kg, even though the mean fetal weights were essentially the same. No significant dose–response effect for fetal weight was evident. Thus, it is concluded that there is no apparent treatment-related effect on fetal weights. No fetal abnormalities, considered related to treatment, were observed (Table 4).

Propionitrile. At the 80-mg/kg dosage level, a single dam died during the study period and a moderate reduction was observed in adjusted mean weight gain (Table 1). As compared to the vehicle control group, there were no dif-

TABLE 4

INCIDENCE OF FETAL ALTERATIONS IN THE RAT FOLLOWING ADIPONITRILE EXPOSURE

	Adiponitrile (mg/kg/day)[a]			
	0	30	50	80
	No. fetuses examined/No. litters examined			
External exam	293/22	296/21	281/22	297/21
Visceral exam	146/22	148/21	143/22	147/21
Skeletal exam	146/22	148/21	138/22	150/21
	No fetuses (No. litters) affected			
External exam				
Short lower jaw	1 (1)	0	0	0
Tarsal flexure	0	0	0	1 (1)
Soft tissue exam				
Malformations				
Hydrocephaly	1 (1)	0	0	0
Retroesophageal aortic arch	0	0	0	1 (1)
Developmental/genetic				
Major vessel variation	0	1 (1)	2 (2)	0
Renal papilla, undeveloped	7 (5)	4 (4)	3 (3)	6 (4)
Skeletal exam				
Malformations				
Scoliosis	0	0	1 (1)	0
Developmental/genetic				
Vertebrae, extra	0	0	0	7 (4)
Rib, rudimentary	40 (14)	32 (12)	31 (13)	46 (15)
Rib, full 14th	0	0	1 (1)	1 (1)
Rib, 13th full/rudimentary	0	0	1 (1)	0
Skull, reduced ossification	0	2 (2)	2 (1)	1 (1)
Hyoid, unossified	1 (1)	5 (2)	0	0
Pubis, unossified	0	0	1 (1)	0
Sternebrae, unossified (5th and 6th)	19 (13)	25 (12)	14 (9)	31 (14)
Sternebrae, unossified (other)	0	4 (3)	4 (3)	0
Sternebrae, misaligned	0	1 (1)	0	0

[a] Administered on gestation Days 6–15. No significant differences from control values were observed.

ferences in the mean number of viable fetuses, resorptions, postimplantation loss, or total implantations in the 20- and 40-mg/kg groups. In the 80-mg/kg/day group, statistically significant ($p < 0.05$) increases were seen in the number of early resorptions as exhibited by an increased incidence of postimplantation loss (Table 2). Mean fetal body weight was significantly ($p < 0.01$) reduced in the 80-mg/kg/day group. These reductions in fetal weight paralleled the severe maternal toxicity seen at this dose level. Embryotoxicity was evidenced by an increase in unossified sternebrae (5 and 6) in both the 40- and 80-mg/kg groups (Table

TABLE 5

INCIDENCE OF FETAL ALTERATIONS IN THE RAT FOLLOWING PROPIONITRILE EXPOSURE

	Propionitrile (mg/kg/day)[a]			
	0	20	40	80
	No. fetuses examined/No. litters examined			
External exam	326/24	302/22	319/22	272/20
Visceral exam	163/24	152/22	162/22	134/20
Skeletal exam	163/24	150/22	157/22	138/20
	No. fetuses (No. litters) affected			
External exam				
Microphthalmia	1 (1)	0	0	0
Fetal anasarca	1 (1)	0	0	0
Soft tissue exam				
Malformations				
Diaphragmatic hernia	0	1 (1)	1 (1)	0
Abdominal closure defect	1 (1)	0	0	0
Developmental/genetic				
Renal papillae undeveloped	0	2 (2)	0	3 (2)
Skeletal exam				
Malformations				
Scoliosis	1 (1)	0	0	1 (1)
Bent ribs	1 (1)	0	0	0
Developmental/genetic				
Vertebrae, extra	0	1 (1)	0	0
Vertebrae, reduced ossification	1 (1)	0	1 (1)	3 (1)
Rib, rudimentary	15 (9)	22 (7)	17 (9)	21 (8)
Rib, full 14th	0	1 (1)	0	0
Rib, 13th full/rudimentary	0	0	0	1 (1)
Skull, reduced ossification	3 (2)	4 (2)	1 (1)	3 (1)
Hyoid, unossified	2 (1)	0	0	0
Pubis, unossified	0	0	1 (1)	2 (1)
Sternebrae, unossified (5th and 6th)	21 (14)	13 (7)	55 (18)	92 (19)
Sternebrae, unossified (other)	1 (1)	1 (1)	2 (2)	8 (2)
Sternebrae, misaligned	2 (2)	1 (1)	0	0

[a] Administered on gestation Days 6–15. No significant differences were observed from control values in parameters measured.

5). This was attributed to the concomitant maternal toxicity, at least at the 80-mg/kg level.

DISCUSSION

Deaths and significant maternal toxicity, related to compound administration, were observed in the high dosage group in each of the three aliphatic nitrile teratogenicity studies. The degree of toxicity following oral administration to pregnant rats appeared to be similar for PN and ADN while ACN was considerably less toxic.

At the highest level tested, both ACN and PN exhibited embryotoxic effects, characterized by increases in both early resorptions and postimplantation loss. In addition, an increase in sites of incomplete skeletal ossification processes *was* observed in fetuses from dams exposed to PN at 40 and 80 mg/kg, or 275 mg/kg ACN. Since each of these levels produced mortality in dams, it is concluded that the embryotoxicity seen at each of these dosages is a reflection of the maternal toxicity. No embryotoxicity was observed at lower levels of ACN or PN, nor did maternal death occur at these test levels.

No teratogenic effects related to administration of ACN, ADN, or PN, were observed at any dosage level tested. No treatment-related major fetal malformations were observed with the three aliphatic nitriles. These results differ from those reported previously, which used a different exposure route and animal model (Willhite *et al.*, 1981; Doherty *et al.*, 1983). In the latter studies, a series of nitriles, including PN, produced cranioschisis occulta and cranioschisis aperta in the fetal hamster after a single exposure by intraperitoneal injection. In all cases, terata occurred only at test levels correlated with significant maternal toxicity. It has been suggested that these teratogenic effects may have been the result of *in vivo* release of cyanide or its metabolite thiocyanate (Willhite *et al.*, 1981). Support for the possible involvement of metabolically released cyanide in the production of terata in hamsters can be found in the limited protective effect offered hamster fetuses when dams dosed with succinonitrile were pretreated with sodium thiosulfate (Doherty *et al.*, 1983). However, free cyanide in substantial concentrations has been detected in tissues from rats exposed acutely to several aliphatic nitriles, including acrylonitrile (AN), PN, and ACN (Ahmed and Farooqui, 1982). Of this group, only AN has induced terata in rats (Murray *et al.*, 1979). Thus, it would appear the results attributed to AN are unique and conversions of these nitriles to cyanide is not a critical factor for production of terata in rats. However, AN (or its reactive metabolite), unlike any other of a series of aliphatic nitriles, is capable of producing irreversible reactions with cellular sulfhydryls or other nucleophiles in rat tissue following acute exposure (Ahmed and Farooqui, 1982). Thus, the teratogenic difference observed between AN and other members of this chemical class could be associated with selectivity in biochemical reactivity or metabolic alteration of the individual nitrile moiety.

On the basis of the present and published literature, no terata have been observed at or below experimental levels of ACN, ADN, and PN which produced maternal toxicity. Thus, these three nitriles are not likely to be a threat to the offspring of pregnant individuals even in the industrial setting.

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