The Effects of Disulfiram on the Experimental C_3H Mouse Embryo

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Disulfiram, in 1 mg and 10 mg oral doses, was given to inbred C_3H mice prior to and for the duration of pregnancy. The effects on the fetus have been assessed at 18 days gestation by means of fetal and placental weights, number of resorptions, skeletal preparations and Wilson's sections. Disulfiram dissolved in diethyl ether was administered to 8- and 9-day embryos *in vitro* in concentrations of 0.1, 10 and 100 $\mu g\ ml^{-1}$ of culture medium, and the effects of ether alone and ether plus disulfiram assessed by evaluation of morphological development over a 28-h period, and inhibition of DNA synthesis using tritiated thymidine labelling over a 4-h period. Disulfiram (1 mg) *in vivo* caused no adverse effects on the fetus, but disulfiram (10 mg) was toxic, in that it caused a significant increase in early resorptions. Disulfiram *in vitro*, in the 10 and 100 $\mu g\ ml^{-1}$ concentrations, proved to be very toxic to the embryos, affecting both morphological development and DNA synthesis in 9-day embryos and morphological development in 8-day embryos. DNA synthesis was only inhibited at the 100 $\mu g\ ml^{-1}$ concentration in 8-day embryos. The 0.1 $\mu g\ ml^{-1}$ concentration of disulfiram caused abnormal central nervous system development in 8-day embryos, but was otherwise non-toxic to 8- and 9-day embryos. Apart from a reduction in somite counts, ether in concentrations of 0.285 mg ml^{-1} and 2.85 mg ml^{-1} caused no adverse effects on morphological development in 8- or 9-day embryos. DNA synthesis was inhibited by ether in a concentration of 2.85 mg ml^{-1} in 9-day embryos.

INTRODUCTION

Disulfiram has been linked with teratogenicity in the human fetus[1] and in experimental Sprague-Dawley rats.[2] In the former, multiple anomalies, including radial aplasia, vertebral fusion, phocomelia of the lower extremities and tracheo-oesophageal fistula, have been described in association with the drug; in rats, there was an increase in resorption rate in dams treated with disulfiram during pregnancy. It was suggested in the latter that the teratogenicity of the agent may be on the basis of induced copper deficiency due to copper chelating activity.

This study was suggested by our previous conclusions that ethanol and acetaldehyde may be teratogenic in the C_3H mouse embryos *in vivo* and *in vitro*.[3] The study was designed to detect whether disulfiram teratogenicity could be confirmed in this animal species, and if so whether it is independent of ethanol and its main metabolite acetaldehyde.

METHODS

Experimental mice

Inbred female C_3H mice were mated with C_3H males at a fixed time. The animals were housed individually in the 1 mg disulfiram *in vivo* group, and in communal cages in the 10 mg and *in vitro* groups. Ambient temperature and light-dark intervals were controlled.

In vivo study

Disulfiram dispergettes containing 400 mg disulfiram as the only active ingredient (supplied by Adcock Ingram Laboratories, Johannesburg) were dissolved in water and administered in a nutritionally balanced liquid feed[4] in a dose of 1 mg, 5 days a week for at least 3 weeks prior to pregnancy and for the duration of gestation. The dose of 1 mg was selected as being approximately 1/400 the lower recommended daily dose of disulfiram in humans. A comparable control group received liquid feed alone. In addition to the liquid feed, the animals had access to rat cubes[4] (Epol Cape Town) and water *ad libitum*. They were weighed once weekly for the duration of the experiment.

A 10 mg dose of disulfiram was administered to a second group via an orogastric tube. The control animals received 1 ml of water in place of the disulfiram. It was decided to increase doses on a logarithmic scale; hence, the choice of the 10 mg dose. An attempt was made to administer the disulfiram as described above, but this was not possible, as it tended to sediment out and block the feeding tubes. The animals were sacrificed on day 18 of gestation (day 1 was regarded as the day following mating). Uteri were removed intact and examined for resorptions, and the fetuses were inspected for obvious abnormalities.

Fetuses and placentas were weighed independently, fixed in 10% saline-buffered formalin, and subsequently divided arbitrarily into two groups for sectioning according to Wilson's method[5] and detailed skeletal examination by a modification of Dawson's staining method,[6] respectively. Histological examination was performed on most fetuses,

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Table 1. ***In vivo*** **study — Effects of chronic disulfiram dosage on fetal development**[a]

Group	Wilson's sections (normal)	Heart sections		Skeletal examination	
		Normal	Abnormal	Normal	Abnormal
Disulfiram (1 mg)	Male 25 Female 19 Total 44	37	1	31	4 Cervical vertebral bodies invivible: 4
Control	Male 29 Female 33 Total 62	59	0	52	a Invisible or poorly developed supraoccipital bone: lone defect: 0 b Cervical vertebral bodies invisible: 1 c Invisible rib (left 9th rib): 1 d Invisible or poorly developed sternal bones: lone defect: 0 e Combined defects — a, b and d above: 2
Disulfiram (10 mg)	Male 24 Female 17 Total 41	30	2	9	a Invisible or poorly developed sternal bones: lone defect: 0 b Invisible or poorly developed supraoccipital bone: lone defect: 0 c Cervical vertebral bodies invisible: 10 d Combined defect — a, b and c above: 2 e Combined defect — b and c above: 4
Control	Male 21 Female 21 Total 42	39	2	27	a Invisible or poorly developed supraoccipital bone: lone defect: 0 b Cervical vertebral bodies invisible: 3 c Invisible or poorly developed sternal bones: 1 d Combined defect — b and c above: 1 e Combined defect — a, b and c above: 1 f Combined defect — a and b above: 6

[a] The 1 mg disulfiram group consisted of 14 dams and 83 live fetuses, and the control group of 18 dams and 118 live fetuses. In the 10 mg disulfiram study, there were 13 dams and 66 live fetuses in the test group, and 12 dams and 81 live fetuses in the control group. Fetuses were assessed at 18 days of gestation. Only live fetuses were included.

Table 2. ***In vitro*** **study groups**[a]

Group	Rat serum (100%)	Disulfiram (μg per ml serum)[b]	Diethyl ether (mg per ml serum)[c]
1	+	–	–
2	+	–	2.85
3	+	–	0.285
4	+	0.1	0.285
5	+	10.0	0.285
6	+	100.0	2.85

[a] The 0.285 mg ml^{-1} ether group served as control for the 0.1 μg ml^{-1} and 10 μg ml^{-1} disulfiram groups, and the 2.85 mg ml^{-1} ether groups as control for the 100 μg ml^{-1} disulfiram group. The embryos cultured in pure rat serum served as controls for the embryos cultured in ether.

[b] Disulfiram, pure substance supplied by MPS Laboratories, Johannesburg.

[c] Diethyl ether, analytical grade, Merck Darmstadt.

which on sectioning appeared abnormal. Fetal hearts were examined in those fetuses used for Wilson's sectioning by a method of microdissection,[4] which enabled detailed examination to be carried out of the atria, atrial appendages, ventricles, aortic and pulmonary valves, coronary arteries and major vessels. The numbers of animals in the test and control groups are indicated in Table 1.

Fetuses were regarded as normal if no abnormalities were detected at 18 days by the criteria referred to above. The skeleton was not considered abnormal at 18 days if the digits were not visible, provided all other bones were clearly identified.

In vitro study

An *in vitro* mouse embryo culture system, based on the method described by New,[7] was used in this investigation. The method was as we have described previously,[3] except that sterile heat-inactivated rat serum was used as the culture medium and plastic tubes were used instead of glass. Eight and 9-day embryos were divided into six groups as indicated in Table 2. There were 10 eight-day embryos and 22 nine-day embryos in group 1. The number of embryos in the remaining groups is indicated in Tables 3 and 4.

DNA synthesis

This was examined in the manner we have reported previously.[3]

Morphological assessment

This was carried out for 8- and 9-day embryos in the manner which we have reported previously.[3]

RESULTS

The detailed results of *in vivo* administration of 1 and 10 mg disulfiram, respectively, are given in Table 1. In the lower dose, there was no significant reduction in fetal or placental weights, or increase in resorption rate, intrauterine deaths, frequency of external or skeletal abnormalities, or alteration in sex ratio compared with controls.

Table 3. ***In vitro*** **study – Effects of disulfiram on 8-day mouse embryos**[a]

Dose of disulfiram ($\mu g\ ml^{-1}$)	Heart – Present – Absent	Heart beat – Present – Absent	Somite count	Neural tube defects	CNS development	DNA synthesis[b]
0.1	No significant effect on development Control: $n = 16$ Test: $n = 16$	No significant effect on heart beat Control: $n = 16$ Test: $n = 16$	No significant effect (Mann-Whitney Test) Control: $n = 15$ Test: $n = 15$	No significant increase in neural tube defects Control: $n = 16$ Test: $n = 16$	CNS development significantly abnormal ($p = 0.042$) Control: $n = 16$ Test: $n = 16$	No significant reduction in DNA synthesis in test group (Mann-Whitney Test) Control: $n = 15$ Test: $n = 15$
10	Significant adverse effect on heart development in test group ($p < 0.001$) Control: $n = 16$ Test: $n = 17$	Significant adverse effect on heart beat in test group ($p < 0.001$) Control: $n = 16$ Test: $n = 17$	Somite count significantly reduced in test group[c] ($p < 0.001$) (Mann-Whitney Test) Control: $n = 16$ Test: $n = 17$	Significant increase in neural tube defects in test group ($p < 0.001$) Control: $n = 16$ Test: $n = 17$	CNS development significantly abnormal ($p < 0.001$) Control: $n = 16$ Test: $n = 17$	No significant reduction in DNA synthesis in test group (Mann-Whitney Test) Control: $n = 15$ Test: $n = 16$
100	Significant adverse effect on heart development in test group ($p < 0.001$) Control: $n = 20$ Test: $n = 17$	Significant adverse effect on heart beat in test group ($p < 0.001$) Control: $n = 20$ Test: $n = 17$	Somite count significantly reduced in test group[c] ($p < 0.001$) (Mann-Whitney Test) Control: $n = 20$ Test: $n = 17$	Significant increse in neural tube defects in test group ($p < 0.001$) Control: $n = 20$ Test: $n = 17$	CNS development significantly abnormal ($p < 0.001$) Control: $n = 20$ Test: $n = 17$	Significant reduction of DNA synthesis in test group ($p < 0.05$) (Mann-Whitney Test) Control: $n = 19$ Test: $n = 15$

[a] The Fisher Exact Probability Test was used, except where otherwise indicated.
[b] Detailed results given in Table 5.
[c] No somites were visible in any of the test embryos in the 10 and 100 $\mu g\ ml^{-1}$ disulfiram groups.

Table 4. *In vitro* **study – Effects of disulfiram on 9-day mouse embryos**[a]

Dose of disulfiram ($\mu g\ ml^{-1}$)	Somite count	Heart beat— Present — Absent	Development of visceral arches	CNS development	DNA synthesis[b]	Neural tube defects
0.1	No significant effect (Mann-Whitney Test) Control: $n = 25$ Test: $n = 26$	No significant effect on heart beat Control: $n = 26$ Test: $n = 25$	No significant abnormality Control: $n = 26$ Test: $n = 25$	CNS development normal Control: $n = 26$ Test: $n = 25$	No significant reduction in DNA synthesis in test group (Mann-Whitney Test) Control: $n = 25$ Test: $n = 24$	No significant increase in neural tube defects Control: $n = 26$ Test: $n = 25$
10	Somite count significantly reduced in test group[c] ($p < 0.001$) (Mann-Whitney Test) Control: $n = 26$ Test: $n = 21$	Significant adverse effect on heart beat in test group ($p = 0.05$) Control: $n = 26$ Test: $n = 24$	Significant abnormality in test group ($p = 0.009$) Control: $n = 26$ Test: $n = 24$	CNS development significantly abnormal in test group ($p < 0.001$) Control: $n = 26$ Test: $n = 24$	Significant reduction in DNA synthesis in test group ($p < 0.001$) (Mann-Whitney Test) Control: $n = 25$ Test: $n = 21$	Significant increase in neural tube defects in test group ($p < 0.001$) Control: $n = 26$ Test: $n = 24$
100	Somite count significantly reduced in test group[c] ($p < 0.001$) (Mann-Whitney Test) Control: $n = 24$ Test: $n = 5$	Significant adverse effect on heart beat in test group ($p < 0.001$) Control: $n = 24$ Test: $n = 25$	Significant abnormality in the test group ($p < 0.001$) Control: $n = 24$ Test: $n = 25$	CNS development significantly abnormal in test group ($p < 0.001$) Control: $n = 24$ Test: $n = 25$	Significant reduction in DNA synthesis in test group ($p = 0.001$) (Mann-Whitney Test) Control: $n = 24$ Test: $n = 20$	Significant increase in neural tube defects in test group ($p < 0.001$) Control: $n = 24$ Test: $n = 25$

[a] The Fisher Exact Probability Test was used, except where otherwise indicated. The development of limb buds, optic and otic vesicles, and the degree of turning of the embryos was very significantly affected ($p < 0.001$) in the two higher disulfiram dose groups studied. Heart development was not affected in any of the groups.

[b] Details given in Table 6.

[c] Only embryos in which it was possible to count somites were included. The remainder were too deformed to assess.

Table 5. *In vitro* **model – Tritiated thymidine uptake of embryos explanted at 8-days gestation**[a]

Rat serum 100% (dpm)	Diethyl ether 2.85 mg ml^{-1} (dpm)	Diethyl ether 0.285 mg ml^{-1} (dpm)	0.1 μg ml^{-1} disulfiram in diethyl ether (dpm)	10.0 μg ml^{-1} disulfiram in diethyl ether (dpm)	100.0 μg ml^{-1} disulfiram in diethyl ether (dpm)
308.0	0	0	980.5	0	157.3
	0	0	566.8	0	0.5
679.4	0	0.5	38.1	73.8	0
892.2	0	529.0	365.8	0.6	297.7
0	0.5	0.5	432.5	0	36.8
469.9	0	629.9	321.0	0	128.3
0	465.7	247.6	673.6	0	69.0
503.0	318.2	0	487.5	0	126.6
37.2	314.4	1671.7	200.1	0	0.5
0	0.5	65.3	752.6	0	149.6
	209.9	757.2	0.5	0.5	289.3
	0	1485.3	1202.5	0	211.2
	344.6	1045.4	639.2	644.7	215.5
	342.6	0	1272.2	0	172.6
	1269.2	1117.3	2453.7	0.5	293.4
	146.5				273.4
	1685.8				
	1627.7				
	773.7				

[a] Taken as reflecting DNA synthesis (Ref. 4).

Table 6. *In vitro* **model – Tritiated thymidine uptake of embryos explanted at 9-days gestation**[a]

Rat serum 100% (dpm)	Diethyl ether 2.85 mg ml^{-1} (dpm)	Diethyl ether 0.285 mg ml^{-1} (dpm)	0.1 μg ml^{-1} disulfiram in diethyl ether (dpm)	10 μg ml^{-1} disulfiram in diethyl ether (dpm)	100 μg ml^{-1} disulfiram in diethyl ether (dpm)
15 048.6	0	16 368.7	12 094.7	372.8	0.5
12 656.3	80.2	14 452.7	25 572.5	1134.0	0
13 785.6	0	0.6	4446.4	0.6	105.1
28 846.2	0	1209.2	24 298.4	363.2	70.2
30 836.4	883.2	6365.9	14 529.0	1019.0	0.5
18 267.1	1696.1	7019.1	14 529.0	797.0	57.6
30 643.4	569.4	8874.3	2150.7	0	0
12 912.8	758.1	12 357.3	9943.5	695.6	72.1
5768.3	918.8	4100.4	15 936.3	529.7	0.5
19 449.2	1873.2	967.8	5388.2	509.3	227.8
10 039.1	0	13 601.7	5539.6	1016.6	0.6
5281.0	9907.4	7987.6	12 627.5	0	154.1
5305.2	7731.2	23 371 4	17 147.0	41.9	131.8
15 128.1	16 287.8	26 347.8	21 608.8	3978.1	102.6
8412.1	5849.5	16 245.1	19 556.9	3169.6	176.1
22 830.0	7528.2	17 266.6	7186.5	2940.7	0
21 182.5	4143.8	16 340.1	8676.8	916.4	0
10 916.8	7368.9	9286.6	0	1711.1	0
7261.1	8803.8	153.1	0.5	776.9	0
4738.2	21 402.2	21 035.2	10 389.2	2424.9	
4628.9	6326.8	8284.6	8806.0	405.7	
	9334.4	30 723.5	7465.4		
	4233.1	4553.2	6762.5		
		6525.6	12 341.2		
		1452.7			

[a] Taken as reflecting DNA synthesis (Ref. 4).

Table 7. Maternal and fetal details relating to C_3H mice treated with 1 mg disulfiram daily, and their controls[a]

Fetal parameters (test group)						Fetal parameters (control group)					
Litter number	Number of fetuses	Range of fetal weights (g)	External abnormality or death	Early resorptions	Range of placental weights (g)	Litter number	Number of fetuses	Range of fetal weights (g)	External abnormality or death	Early resorptions	Range of placental weights (g)
1	7	0.8932-1.01185 Mean 1.0241 ± 0.0795	–	1	0.1188-0.1565 Mean 0.1409 ± 0.0155	1	10	0.9583-1.1436 Mean 1.0336 ± 0.0554	One monster or late resorption	0	0.0885-0.1531 Mean 0.1085 ± 0.0182
2	7	0.6570-1.1012 Mean 0.9052 ± 0.1486	–	0	0.1074-0.1803 Mean 0.1469 ± 0.0282	2	7	0.6085-1.2235 Mean 1.0929 ± 0.2182	–	1	0.1077-0.1703 Mean 0.1385 ± 0.0213
3	8	0.9045-1.0672 Mean 0.9696 ± 0.0495	–	2	0.1433-0.2342 Mean 0.1719 ± 0.0278	3	6	0.9505-1.1905 Mean 1.1030 ± 0.0906	One monster or late resorption	2	0.1111-0.1801 Mean 0.1442 ± 0.0247
4	10	0.8697-1.0375 Mean 0.9756 ± 0.0525	–	0	0.1283-0.2245 Mean (twins) 0.1546 ± 0.0295	4	5	1.1242-1.1755 Mean 1.1508 ± 0.0236	–	0	0.1230-0.1700 Mean 0.1332 ± 0.0206
5	7	0.8292-1.0246 Mean 0.9454 ± 0.0627	–	2	0.1095-0.1972 Mean 0.1416 ± 0.0272	5	10	0.8813-1.0727 Mean 0.9711 ± 0.0666	–	1	0.0900-0.1400 Mean 0.1073 ± 0.0134
6	6	0.8051-1.2123 Mean 1.0168 ± 0.1391	–	0	0.1112-0.1649 Mean 0.1413 ± 0.0201	6	9	0.7956-1.0654 Mean 0.9459 ± 0.0763	–	0	0.0765-0.1320 Mean 0.1130 ± 0.0174
7	8	0.9350-1.1162 Mean 1.0512 ± 0.0682	One monster or late resorption	0	0.1158-0.1383 Mean 0.1269 ± 0.0091	7	9	0.7895-0.9800 Mean 0.8824 ± 0.0777	–	0	0.0915-0.1517 Mean 0.1127 ± 0.0184
8	7	0.9796-1.1500 Mean 1.0546 ± 0.0587	One monster or late resorption	1	0.1199-0.1826 Mean 0.1581 ± 0.0256	8	8	0.6039-1.1662 Mean 0.9974 ± 0.1829	One monster or late resorption	0	0.0820-0.1263 Mean 0.1070 ± 0.0143
9	7	0.6469-1.0972 Mean 0.9712 ± 0.1641	Abnormal hind limb	2	0.0678-0.1123 Mean 0.0962 ± 0.0166	9	10	0.8562-1.0434 Mean 0.9565 ± 0.0641	–	0	0.0781-0.1111 Mean 0.0978 ± 0.0115

10	6	0.6930–1.1684 Mean 0.9557 ± 0.1818	–	1	0.0893–0.2234 Mean (twins) 0.1319 ± 0.0533	10	8	0.9802–1.2123 Mean 1.0860 ± 0.0781	One monster or late resorption and one dead fetus	1	0.1013–0.3167 Mean (twins) 0.1776 ± 0.0755
11	8	0.8400–1.1300 Mean 1.0533 ± 0.0907	–	0	0.0900–0.1338 Mean 0.1086 ± 0.0128	11	9	0.7320–1.1124 Mean 0.9666 ± 0.1258	One monster or late resorption	1	0.1022–0.2121 Mean (twins) 0.1479 ± 0.0378
12	9	0.9334–1.1494 Mean 1.0364 ± 0.0707	One monster or late resorption	1	0.0887–0.1225 Mean 0.1066 ± 0.0124	12	10	0.8760–1.1309 Mean 1.0083 ± 0.9760	One dead fetus	0	0.1144–0.1596 Mean 0.1377 ± 0.0165
13	9	0.9141–1.2004 Mean 1.0673 ± 0.0827	One monster or late resorption and one dead fetus with exomphalos	0	0.0942–0.2298 Mean 0.1202 ± 0.0487	13	6	0.7014–1.1600 Mean 0.9778 ± 0.1637	–	2	0.1019–0.2062 Mean 0.1566 ± 0.0360
14	10	0.8828–1.0624 Mean 0.9842 ± 0.0623	One dead fetus	0	0.0897–0.1282 Mean 0.1050 ± 0.0114	14	8	0.9990–1.1614 Mean 1.0772 ± 0.0758	One monster or late resorption and one dead fetus	2	0.0961–0.1693 Mean 0.1412 ± 0.0313
						15	8	0.7293–1.0359 Mean 0.9311 ± 0.0935	One fetus with exomphalos	0	0.1257–0.3135 Mean (twins) 0.1795 ± 0.0630
						16	7	0.8300–1.1269 Mean 1.0025 ± 0.0926	–	0	0.0832–0.1130 Mean 0.1056 ± 0.0101
						17	8	0.9090–1.0343 Mean 0.9696 ± 0.0502	–	3	0.0851–0.1061 Mean 0.0946 ± 0.0079
						18	9	0.9440–1.0895 Mean 1.0292 ± 0.0499	One monster or late resorption	1	0.1000–0.1800 Mean 0.1329 ± 0.0263

[a] Late resorptions and monsters have been included as intrauterine deaths. The pre-pregnant weight of the dams in the 1 mg test group ranged from 18.2 to 29.0 g (mean 23.2 g), and in the control group from 22.4 to 29.5 g (mean 25.8 g). The mean duration of the experiment in the test group ranged from 32 to 41 days (mean 35.7 days), and in the control group from 35 to 55 days (mean 45.7 days). In every case, the pre-pregnant and pregnant state of the dams was examined and confirmed to be healthy. The mean daily maternal dietary intake of the liquid food ranged from 4.07 to 4.81 ml in the test animals and from 4.84 to 5.0 ml in the control animals.

Table 8. Maternal and fetal details relating to C_3H mice treated with 10 mg disulfiram daily, and their controls[a]

Fetal parameters (test group)						Fetal parameters (control group)					
Litter number	Number of fetuses	Range of fetal weights (g)	External abnormality or death	Early resorptions	Range of placental weights (g)	Litter number	Number of fetuses	Range of fetal weights (g)	External abnormality or death	Early resorptions	Range of placental weights (g)
1	5	1.2810–1.3525 Mean 1.3230 ± 0.0307	–	3	0.0845–0.1114 Mean 0.0991 ± 0.0133	1	8	0.5189–0.7480 Mean 0.6439 ± 0.0755	–	0	0.1010–0.1355 Mean 0.1179 ± 0.0133
2	8	0.6070–0.8066 Mean 0.7139 ± 0.0627	–	0	0.1013–0.1574 Mean 0.1237 ± 0.0216	2	9	0.5132–0.8136 Mean 0.6869 ± 0.1177	–	1	0.0968–0.1357 Mean 0.1148 ± 0.0153
3	9	0.7905–1.0246 Mean 0.9176 ± 0.0750	–	0	0.0695–0.1095 Mean 0.0949 ± 0.0120	3	6	0.9944–1.0326 Mean 1.0479 ± 0.0451	–	1	0.0900–0.1538 Mean 0.1164 ± 0.0221
4	6	0.7334–0.9508 Mean 0.8580 ± 0.0979	Monster or late resorption	0	0.0952–0.1519 Mean 0.1137 ± 0.0223	4	6	0.7027–1.0647 Mean 0.9457 ± 0.1285	–	0	0.0943–0.1630 Mean 0.1369 ± 0.0279
5	7	0.8101–1.1578 Mean 0.9803 ± 0.1176	–	0	0.1410–0.1791 Mean 0.1560 ± 0.0158	5	7	0.9414–1.1119 Mean 0.9833 ± 0.0770	One dead fetus	0	0.1200–0.1439 Mean 0.1296 ± 0.0083
6	5	0.9048–1.0186 Mean 0.9387 ± 0.0536	Monster or late resorption	2	0.1315–0.2300 Mean 0.1688 ± 0.0432	6	7	0.9353–1.0610 Mean 0.9933 ± 0.0447	–	0	0.1118–0.1741 Mean 0.1360 ± 0.0201

7	5	0.8675–0.9788 Mean 0.9373 ± 0.0529	Monster or late resorption	0	0.1168–0.1691 Mean 0.1516 ± 0.0236	7	6	0.8963–1.0807 Mean 1.0134 ± 0.0695	–	0	0.1073–0.2708 Mean 0.1519 ± 0.0678
8	5	0.8999–1.2110 Mean 1.0449 ± 0.1162	–	1	0.1075–0.1460 Mean 0.1242 ± 0.0174	8	6	0.9423–1.0289 Mean 1.0048 ± 0.0336	–	1	0.1211–0.1505 Mean 0.1369 ± 0.0114
9	5	0.7398–0.8756 Mean 0.8468 ± 0.0599	–	3	0.1159–0.1483 Mean 0.1347 ± 0.0128	9	4	0.9218–1.0928 Mean 1.0089 ± 0.0725	–	0	0.1328–0.1779 Mean 0.1523 ± 0.0191
10	5	0.7475–0.9928 Mean 0.8999 ± 0.1006	–	3	0.0855–0.1540 Mean 0.1290 ± 0.0259	10	6	0.8961–1.0789 Mean 0.9998 ± 0.0660	–	0	0.1054–0.1495 Mean 0.1278 ± 0.0171
11	8	0.6911–0.9759 Mean 0.8674 ± 0.0862	–	0	0.0965–0.1382 Mean 0.1274 ± 0.0140	11	7	0.9059–1.0766 Mean 0.9801 ± 0.0648	–	0	0.1129–0.2265 Mean (twins) 0.1479 ± 0.0402
12	6	0.8243–0.9900 Mean 0.9149 ± 0.0638	Monster or late resorption	2	0.1091–0.1510 Mean 0.1236 ± 0.0181	12	9	0.7962–1.0596 Mean 0.9187 ± 0.0891	–	0	0.1063–0.1368 Mean 0.1234 ± 0.0114
13	3	0.8494–1.0599 Mean 0.9656 ± 0.1070	–	2	0.1081–0.1847 Mean 0.1464 ± 0.0383						

[a] Late resorptions and monsters have been included in intrauterine deaths. In every case, the pre-pregnant and pregnant state of the dams was examined and confirmed to be healthy.

In the 10 mg dose, disulfiram exerted no detectable effect on any of these parameters, besides the resorption rate, which was significantly increased. Late resorptions or monsters were included in intra-uterine deaths.

The results of the *in vitro* studies of 8- and 9-day embryos are given in Tables 3 and 4, respectively. The data concerning group 1 which served as an internal control for groups 2 and 3 has not been included in the tables, as they did not differ materially. The detailed results of tritiated thymidine uptake in the various groups are given in Tables 5 and 6.

Disulfiram in low dosage caused central nervous system abnormalities in 8-day embryos, but no other abnormalities were detected in either 8- or 9-day embryos at this dosage. Concentrations of 10 and 100 $\mu g\ ml^{-1}$ disulfiram proved to be highly toxic to both 8- and 9-day embryos, affecting most of the criteria which were examined.

DNA synthesis was significantly reduced by the highest dose of disulfiram in 8-day embryos. In the 9-day-old embryos, DNA synthesis was inhibited in the 10 and 100 $\mu g\ ml^{-1}$ disulfiram groups.

In the concentrations studied, diethyl ether caused no detectable morphological abnormalities in 8- or 9-day embryos besides a reduction in the somite counts in 8- and 9-day embryos in the higher concentration ($p = 0.05$ and 0.003, respectively).

Neither concentration of diethyl ether had an effect on DNA synthesis of 8-day embryos. DNA synthesis was inhibited by 2.85 mg of diethyl ether per ml in 9-day embryos ($p = 0.0002$). This finding was associated with a reduction in somite count, but not with other detectable morphological abnormalities.

Two fetuses in the 10 mg disulfiram control group had abnormal hearts. In one there was a dilated right ventricle, and in the other a small heart was noted, with the aorta and pulmonary artery abnormally approximated. The origin of the pulmonary artery in this heart was anterior to its normal position.

In the group treated with 10 mg disulfiram, one fetus had a small aorta and dilated pulmonary artery, and the other had a small heart associated with what appeared to be a truncus arteriosus.

Further details regarding the condition of the dams in their pre-pregnant and pregnant state, and fetal weight, frequency of abnormalities, deaths or resorptions and placental weight of test and control animals are provided in Tables 7 and 8.

DISCUSSION

The high dose of disulfiram (10 mg daily) was associated with a significant increase in resorptions, but not with any other identifiable morphological abnormalities in those fetuses which survived. Possible disulfiram-induced biochemical or functional abnormalities were not examined in this study, and cannot be excluded.

The *in vitro* findings suggest a teratogenic effect of disulfiram in high doses. The findings of a reduction of DNA synthesis in 8- and 9-day embryos with the higher doses of disulfiram were not consistently related to morphological evidence of abnormal central nervous system development. Our previous experience (with larger numbers) gave good correlation between inhibition of DNA synthesis and abnormal central nervous system development caused by two mouse teratogens, ethanol and acetaldehyde.

Our conclusion is that disulfiram, given in high pharmacological doses to C_3H mice prior to and during pregnancy, exerts a teratogenic effect. There was an indication that this toxic effect of disulfiram is dose-related.

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