Embryo Toxicity and Teratogenicity of Formaldehyde

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ABSTRACT. C-14 formaldehyde crosses the placenta and enters fetal tissues. The incorporated radioactivity is higher in fetal organs (i.e., brain and liver) than in maternal tissues. The incorporation mechanism has not been studied fully, but formaldehyde enters the single-carbon cycle and is incorporated as a methyl group into nucleic acids and proteins. Also, formaldehyde reacts chemically with organic compounds (e.g., deoxyribonucleic acid, nucleosides, nucleotides, proteins, amino acids) by addition and condensation reactions, thus forming adducts and deoxyribonucleic acid-protein crosslinks. The following questions must be addressed: What adducts (e.g., *N*-methyl amino acids) are formed in the blood following formaldehyde inhalation? What role do *N*-methyl-amino adducts play in alkylation of nuclear and mitochondrial deoxyribonucleic acid, as well as mitochondrial peroxidation? The fact that the free formaldehyde pool in blood is not affected following exposure to the chemical does not mean that formaldehyde is not involved in altering cell and deoxyribonucleic acid characteristics beyond the nasal cavity. The teratogenic effect of formaldehyde in the English literature has been sought, beginning on the 6th day of pregnancy (i.e., rodents) (*Saillenfait AM,* et al. Food Chem Toxicol 1989, pp 545–48; *Martin WJ.* Reprod Toxicol 1990, pp 237–39; *Ulsamer AG,* et al. Hazard Assessment of Chemicals; Academic Press, 1984, pp 337–400; and U.S. Department of Health and Human Services. Toxicological Profile of Formaldehyde; ATSDR, 1999 [references 1–4, respectively, herein]). The exposure regimen is critical and may account for the differences in outcomes. Pregnant rats were exposed (a) prior to mating, (b) during mating, (c) or during the entire gestation period. These regimens (a) increased embryo mortality; (b) increased fetal anomalies (i.e., cryptochordism and aberrant ossification centers); (c) decreased concentrations of ascorbic acid; and (d) caused abnormalities in enzymes of mitochondria, lysosomes, and the endoplasmic reticulum. The alterations in enzymatic activity persisted 4 mo following birth. In addition, formaldehyde caused metabolic acidosis, which was augmented by iron deficiency. Furthermore, newborns exposed to formaldehyde in utero had abnormal performances in open-field tests. Disparities in teratogenic effects of toxic chemicals are not unusual. For example, chlorpyrifos has not produced teratogenic effects in rats when mothers are exposed on days 6–15 (*Katakura Y,* et al. Br J Ind Med 1993, pp 176–82 [reference 5 herein]) of gestation (*Breslin WJ,* et al. Fund Appl Toxicol 1996, pp 119–30; and *Hanley TR,* et al. Toxicol Sci 2000, pp 100–08 [references 6 and 7, respectively, herein]). However, either changing the endpoints for measurement or exposing neonates during periods of neurogenesis (days 1–14 following birth) and during subsequent developmental periods produced adverse effects. These effects included neuroapoptosis, decreased deoxyribonucleic acid and ribonucleic acid synthesis, abnormalities in adenylyl cyclase cascade, and neurobehavioral effects (*Johnson DE,* et al. Brain Res Bull 1998, pp 143–47; *Lassiter TL,* et al. Toxicol Sci 1999, pp 92–100; *Chakraborti TK,* et al. Pharmacol Biochem Behav 1993, pp 219–24; *Whitney KD,* et al. Toxicol Appl Pharm 1995, pp 53–62; *Chanda SM,* et al. Pharmacol Biochem Behav 1996, pp 771–76; *Dam K,* et al. Devel Brain Res 1998, pp 39–45; *Campbell CG,* et al. Brain Res Bull 1997, pp 179–89; and *Xong X,* et al. Toxicol Appl Pharm 1997, pp 158–74 [references 8–15,

respectively, herein]). Furthermore, the terata caused by thalidomide is a graphic human example in which the animal model and timing of exposure were key factors (*Parman T*, et al. Natl Med 1999, pp 582–85; and *Brenner CA*, et al. Mol Human Repro 1998, pp 887–92 [references 16 and 17, respectively, herein]). Thus, it appears that more sensitive endpoints (e.g., enzyme activity, generation of reactive oxygen species, timing of exposure) for the measurement of toxic effects of environmental agents on embryos, fetuses, and neonates are more coherent than are gross terata observations. The perinatal period from the end of organogenesis to the end of the neonatal period in humans approximates the 28th day of gestation to 4 wk postpartum. Therefore, researchers must investigate similar stages of development (e.g., neurogenesis occurs in the 3rd trimester in humans and neonatal days occur during days 1–14 in rats and mice, whereas guinea pigs behave more like humans). Finally, screening for teratogenic events should also include exposure of females before mating or shortly following mating. Such a regimen is fruitful inasmuch as environmental agents cause adverse effects on ovarian elements (e.g., thecal cells and ova [nuclear-deoxyribonucleic acid and mitochondrial deoxyribonucleic acid]), as well as on zygotes and embryos before implantation. Mitochondrial deoxyribonucleic acid mutations and deletions occur in human oocytes and embryos (*Parman T*, et al. Natl Med 1999, pp 582–85; and *Brenner CA*, et al. Mol Human Repro 1998, pp 887–92 [references 16 and 17, respectively, herein]). Thus, it is likely that xenobiotics directly affect n-deoxyribonucleic acid and/or mitochondrial deoxyribonucleic acid in either the ovum or the zygote/embryo or both (*Thrasher JD*. Arch Environ Health 2000, pp 292–94 [reference 18 herein]), and they could account for the increasing appearance of a variety of mitochondrial diseases, including autism (*Lomard L*. Med Hypotheses 1998, pp 497–99; *Wallace EC*. Proc Natl Acad Sci 1994, pp 8730–46; and *Giles RE*, et al. Proc Natl Acad Sci 1980, pp 6715–19 [references 19–21, respectively, herein]). Two cases of human birth defects were reported in formaldehyde-contaminated homes (*Woodbury MA*, et al. Formaldehyde Toxicity 1983; pp 203–11 [reference 22 herein]). One case was anencephalic at 2.76 ppm, and the other defect at 0.54 ppm was not characterized. Further observations on human birth defects are recommended.
<Key words: anomalies, chromosomes, embryo, formaldehyde, mitochondria, mutagenicity, neurogenesis, teratogenicity>

FORMALDEHYDE (FA), which is widely spread in the environment, is used in the manufacture of a wide variety of products, and its major uses are ureaformaldehyde (UF) resins (25%), phenol-FA (PhF) resins (20%), plastics (15%), and intermediates (22%). It is an intermediate in acetylinic chemicals, and it is used to produce 4,4′-hexamethylenetetramine, UF concentrates, 4,4′-methylenediphenyl diisocyanate, chelating agents, and trimethylolpropane. Ureaformaldehyde and PhF resins are used primarily as adhesives in the manufacture of particle board, fiberboard, plywood, and molding, and they are used in paper treating and coating, textile treating, surface coating, and fiberglass insulation.[23] The Occupational Safety and Health Administration (OSHA) estimates that approximately 2.1 million workers are exposed to FA.[23,24] Domestic exposures occur mainly from consumer products, including textiles (clothing and household furnishings), insulation (fibrous and foams), paper, cosmetics, and wood products (e.g., particle board, plywood, medium-density fiber board).[25]

The current OSHA permissible exposure limit is 0.75 ppm (i.e., an 8-hr time-weighted average [TWA]). This standard includes a 2-ppm, short-term, 15-min exposure limit, with an "action level" that is 0.5 ppm measured over 8 hr.[24] Other exposure levels follow: American Conference of Governmental Industrial Hygienists threshold limit value of 0.3 ppm (0.37 mg/m^3 ceiling) and the National Institute of Occupational Safety and Health (NIOSH) recommended exposure limit (REL) of 0.016 ppm (TWA) and 0.1-ppm (15-min ceiling). Formaldehyde is classified as a "probable human carcinogen": the U.S. Environmental Protection Agency (EPA) identifies it as a Class 2A carcinogen; the International Agency for Research on Cancer, a Class 2A carcinogen; OSHA, a carcinogen; NIOSH, a carcinogen; and the National Toxicological Program, reasonably anticipated as a carcinogen.[26]

Acute health effects and doses follow: odor threshold (0.05–1.0 ppm); eye irritation (0.01–2.0 ppm); irritation of eyes, nose, throat, and upper respiratory system (1.0–3.0 ppm); intolerable (4.0–5.0 ppm); severe respiratory symptoms and difficulty breathing (10–20 ppm); serious respiratory tract injury (> 50 ppm); and death (> 100 ppm). The immediately dangerous to life and health level (IDLH) is 20 ppm for a 13–30-min exposure. Chronic exposure to FA can lead to dermal and respiratory sensitization, lower airway and chronic pulmonary obstruction, and immunologic manifestations.[27–29] Evaporation from formalin at 20 °C yields 5 ppm of FA.

Researchers believe that reproductive and developmental effects relative to FA exposure are minimal. This conception is based on a few epidemiological studies conducted before 1989 by EPA[30] and the World Health Organization[31] and on the absence of birth defects in animal studies following FA exposure.[32] More-recent epidemiologic investigations have shown that exposure to FA is associated with delayed conception[33] and an increased risk of spontaneous abortion in woodworkers,[33] laboratory personnel,[34] and cosmetologists.[35] Reports from Japanese and Russian literature on the embryotoxicity of FA in rodents demonstrate that FA

crosses the placenta to the fetus, causes birth defects, and affects enzyme function in the mitochondria, lysosomes, and endoplasmic reticulum (ER). In the current study, we review this research and critique the perspective of the current scientific knowledge of the biological chemistry of FA.

Method and Materials

Distribution of ^{14}C-labeled FA (14CFA) in maternal and fetal tissues. The uptake and distribution of 14CFA were studied in pregnant ICR mice.[5,36,37] On the 16th day of gestation, the tail veins of adult pregnant mice were injected with 0.05 ml of 1% formalin containing 3.5 mg of 14CFA. The animals were killed at intervals from 5 min up to 48 hr. The incorporation of 14CFA and its metabolites was followed by frozen section autoradiography and liquid scintillation detection. We undertook control measures to avoid errors from loss of radioactivity by volatility of the 14CFA and its metabolites from frozen sections. Trichlororacetic acid– treated maternal and fetal liver showed the incorporation of the isotope into deoxyribonucleic acid (DNA) and acid-insoluble fractions.

The autoradiograms and scintillation counting demonstrated a rapid uptake (by 5 min) of 14CFA into maternal liver, lung, heart, salivary gland, gall bladder, spleen kidney, bone marrow, nasal mucosa, uterus, placenta, and fetal tissues (Table 1). The radioactivity appeared in urine and feces up to 6 hr following injection.

Incorporation of the labeled isotope was greater in the placenta, uterus, and fetal tissues than in other maternal organs (Table 1). The fetal brain had significantly greater uptake than the maternal brain at 6 hr after injection. At 48 hr after injection, residual radioactivity in the mother and fetus was 29.6% of the administered dose. The remaining 14CFA was either expired or excreted in urine and feces. The DNA fraction contained 20% and 50% of the total radioactivity in maternal and fetal liver at 6 hr and 24 hr after injection, respectively (Fig. 1).

Elimination of FA and its metabolites from fetal tissues was slower than from maternal tissues. This was particularly evident in fetal liver and brain. Moreover, the radioactivity in the fetal brain was twice that observed in the maternal brain at 6 hr and afterward (Table 1).

Cytopathic and cytogenetic effects of FA inhalation on germ cells and bone marrow cells. Adult female Wistar rats (experimental and control) were housed under controlled lighting (i.e., 12 hr light/12 hr dark) and given free access to food and water.[38] Females were exposed to FA via inhalation 4 hr/day in special chambers for 4 mo (except on nonworking days) at 0.5 and 1.5 mg/m^3 of air. Following exposure, exposed and control females were mated with intact males. Embryos were extracted from uteri by saline solution lavage on days 2 and 3 of pregnancy. The morphology of embryos was observed by MBS-9 and MB1-11 (phase-contrast illumination) microscopy. A portion of the embryos was used for making up total Tartosky preparations.

Bone marrow was taken from the same animals 48–72 hr following termination of FA exposure and was prepared for cytogenetic studies by the standard method. One hundred metaphases were analyzed per animal from coded preparations for the mitotic index. All types of chromosomal aberrations and cells containing from 40–43 chromosomes were recorded (gaps were not included). In addition, the dependence of the frequency of breaks in chromosomes on their lengths was established. The significance of difference in mean values was assessed with Student's *t* test, and differences in proportions was determined with the chi-square test.

Table 1.—Maternal and Fetal Concentrations of Radioactivity (dpm/mg [µl]) in Pregnant Mice (n = 5)

Time after injection		Blood		Brain (whole)		Liver		Placenta		Fetus (whole)		Amniotic fluid		Amnion		Uterus	
		$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*
5 min	Maternal	154.4	39.9	36.7	4.6	307.9	58.8	65.5	27.4			39.2	33.8	56.8	23.8	141.9	37.3
	Fetal			31.8	10.4	88.9	39.3			63.1	30.6						
30 min	Maternal	79.0	13.4	48.7	3.1	269.3	43.3	75.3	22.8			19.3	6.9	53.9	9.9	110.2	29.9
	Fetal			47.7	20.1	129.0	42.5			81.1	19.7						
1 hr	Maternal	117.1	24.1	44.6	0.5	298.2	66.7	89.9	39.7			14.6	7.9	58.4	29.1	109.3	26.5
	Fetal			56.2	34.6	150.3	4.3			85.1	32.0						
3 hr	Maternal	149.4	18.9*	53.1	25.6	361.8	64.5	120.1	23.8			18.8	11.3	80.2	21.1	135.5	27.8
	Fetal			71.0	24.2	263.9	56.7			117.7	26.5						
6 hr	Maternal	48.8	3.8	22.9	6.0	180.4	42.0	62.6	0.11			8.9	4.9	51.1	17.0	82.4	10.4
	Fetal			35.0	8.0†	142.5	38.1			79.5	25.7						
24 hr	Maternal	15.4	5.8	17.7	4.2	105.7	37.7	48.5	21.3			4.9	1.9	45.3	21.1	69.3	20.1
	Fetal			36.5	12.1‡	96.2	38.6‡			65.0	29.0						
48 hr	Maternal	9.0	2.5	9.5	2.5	48.3	10.8	25.9	4.8								
	Fetal			22.3	5.9‡	48.2	16.6										

Notes: $\bar{x}$ = mean, and *SD* = standard deviation.
*$p < .05$; significantly different from radioactivity at 30 min.
†$p < .05$.
‡$p < .01$; significantly different from maternal radioactivity.

No significant effect of 0.5 mg/m³ of FA was observed on embryonic development on the 3rd day of pregnancy. However, at 1.5 mg/m³ there was damage to structure (i.e., roughness of cytoplasm and pyknosis of nuclei) of blastomeres, and there was an increased proportion of degenerating embryos ($p < .05$).

The number of metaphases with chromosome aberrations at 0.5 mg/m³ was significantly higher than in controls ($p < .05$) and was elevated at the higher concentration ($p < .01$) (Table 2). In addition, the number of chromosomes with aberrations and aneuploidy was significantly elevated ($p < .05$). The mitotic index was decreased at 0.5 mg/m³ ($p < .05$) and was increased at 1.5 mg/m³ ($p < .05$), compared with controls.

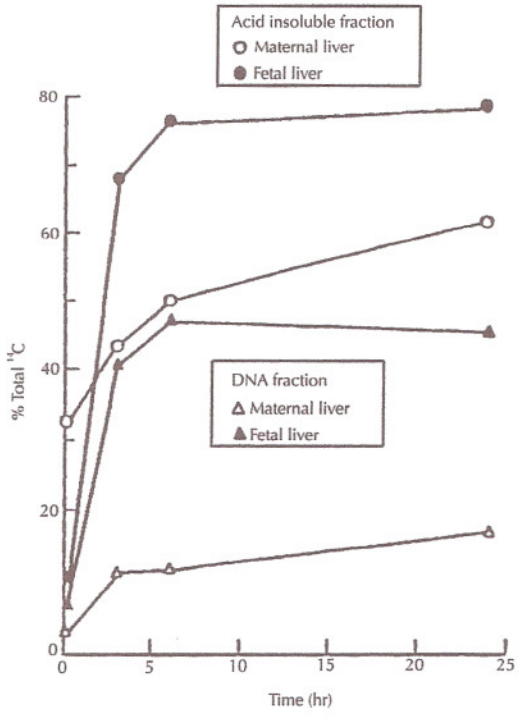

Fig. 1. Binding of ^{14}C from C-formaldehyde to cells and deoxyribonucleic acid (DNA) of maternal and fetal mice livers. The upper 2 lines show percentage of ^{14}C in acid-insoluble fraction (M = mother, F = fetus); the lower 2 lines show percentage of ^{14}C in DNA.

The frequency of chromosome breaks was proportional to length. At the same time, the breaks occurred more frequently in the centromere region (62%) in metacentric chromosomes and in the telomere region (52%) in acrocentric chromosomes. An increase in the frequency of genome mutations (i.e., the number of hypoploid cells) was also observed in experimental rats.

At low doses, FA possesses cytopathogenic and mutagenic effects, and the cells studied had different levels of susceptibility to the harmful effects of this chemical. Bone marrow cells were more sensitive to the effects of FA than were germ cells. Bone marrow cells exhibited chromosome aberrations, aneuploidy, and changes in the mitotic index. The morphological damage detected in the embryos was not specific to FA, inasmuch as such degeneration followed exposure to gasoline and contraceptives (as stated by authors).

It is hypothesized that FA (and possibly many other substances) affects ovarian follicules (i.e., differentiation of follicle somatic cells), leading to a disruption in egg maturation (i.e., final stages of gametogenesis) and to an impairment of fertilization and early embryonic development.

Prolonged inhalation of FA (near maximum allowable concentrations) affects bone marrow, and possibly ovarian elements, zygote, and early embryogenesis; most likely there is impaired proliferative activity and chromosome damage (i.e., aberrations and aneuploidy).

Effects of FA inhalation on ascorbic acid, nucleic acids, organ weights, and pathology in newborn rats. The effects of FA inhalation on several fetal parameters have been reported.[39–41] Female adult rats inhaled FA (0.012 mg/m³ and 1.0 mg/m³) for 10–15 days, after which they joined unexposed males, then mated, and were exposed throughout gestation. Control pregnant females were handled in the same manner with respect to food, temperature, humidity, and velocity of air movement; the only exception was that purified air was supplied to the exposure chamber. The animals were killed at parturition.

Duration of pregnancy was increased by 14–15%, but the number of animals per litter was decreased in

Table 2.—Frequency of Chromosomal Damage and Aneuploidy in Bone Marrow Cells of Rats Exposed to Formaldehyde (FA)

Concentration of FA (mg/m³)	Number of metaphases analyzed	Number of cells with aberrations		Number of aberrations per 100 metaphases				Number of chromosomes with aberrations and aneuploidy				Mitotic index (%)	
				Chromatid		Chromosome		< 42		> 42			
		$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*
Control	600	0.7	0.3	0.3	0.2	0.3	0.2	7.0	1.0	0.2	0.1	5.0	0.3
0.5	785	2.4	0.5*	2.3	0.6	0.2	0.2	10.9	1.1*	0.8	0.3	4.2	0.2*
1.5	625	4.0	0.7†	2.7	0.6*	1.6	0.5*	13.6	1.4*	0.0	0.04	6.7	0.3*

Notes: $\bar{x}$ = mean, and *SD* = standard deviation.
*Differed reliably from the control group: $p < .05$.
†Differed reliably from the control group: $p < .01$.

rats that inhaled 0.012 mg/m^3 (n = 9.8 animals/litter) and 1.0 mg/m^3 (n = 8.6) FA, compared with controls (n = 11.3). Ascorbic acid content of the whole fetus, fetal liver, and maternal liver was decreased, compared with controls (Table 3). In newborns, a statistically significant increase in body weight and weights of thymus, heart, kidney, and adrenals—but a decrease in lung and liver—were found (Table 3). The authors stated that exposure to FA lowered DNA content and increased ribonucleic acid (RNA) content in fetal organs (data not shown). At 1.0 mg/m^3, there was an involution of lymphoid tissues, mild hypertrophy of Kupffer's cells, and numerous extramedullary myelopoietic centers, histochemistry reduced glycogen content of the myocardium and liver, accumulation of positive Schiff's reaction product in the kidney, and presence of iron in Kupffer's cells.

Embryotoxic effects of FA on marker enzymes of intracellular organelles. Pregnant rats received an aqueous solution of 8 mg/kg (1/50 of low dose, 50% fatality [LD_{50}]) of FA intragastrically (once/day) throughout pregnancy until they were killed on the 20th day of gestation.[42] The indices of overall embryo mortality and pre- and postimplantation mortality were calculated, and anatomical defects were recorded. Activity of several enzymes and *N*-acetyl-neuraminic acid concentrations were determined in fetal and liver tissues (Table 4).

Formaldehyde caused a 2-fold increase in pre- and postimplantation mortality and in embryo mortality. There was decreased activity in mitochondrial enzymes, malate dehydrogenase (MDH [decreased by 30% {$p < .05$}]), and succinate dehydrogenase (SDH [decreased by 50% {$p < .05$}]); however, glutamate dehydrogenase activity increased. Formaldehyde also affected mitochondrial innermembrane permeability and oxidative phosphorylation, and energy production was impaired.

Microsomal and lysosomal inosine diphosphatase activity decreased in the embryos (liver) and in females (placenta and liver) by an average of 59% and 42% ($p < .05$), respectively. Beta-glucuronidase activity also decreased by 26% in the liver of the embryo ($p < .05$). Neuraminic acid increased in the liver of the females and in embryos by 28% and 22%, respectively ($p < .05$), and there was a corresponding significant rise in serum concentration (Table 4).

Increased overall embryo mortality was correlated with enzyme activity. The decrease in β-glucuronidase in the fetal liver and the decrease in MDH in the maternal liver were correlated with embryo mortality ($r = -.67$ [$p < .05$] and $r = -.63$ [$p < .05$], respectively). In addition, the increase in *N*-acetyl-neuraminic acid in maternal liver and serum was also correlated with embryo mortality ($r = +.067$ [$p < .05$] and $r = +.94$ [$p < .05$], respectively). Therefore, changes in the activity of marker enzymes of lysosomes, mitochondria, and endoplasmic reticulum of the most important organs and systems reflected embryo mortality.

Effects of prenatal FA exposure on postnatal organ morphology and function. Formaldehyde was administered orally in an aqueous solution at a dose of 0.5 mg/kg · day to female mongrel rats during the 1st–21st days of pregnancy.[43] The authors assessed the embryotoxicity of FA by determining the (a) number(s) of live births for each pregnant rat; (b) number that survived on the 4th and 21st days of life; (c) number that died by the

Table 3.—Effects of Exposure to Formaldehyde (FA) Vapor on Organ Weights of Newborns and Ascorbic Acid Content in Whole Fetus, Placenta, and Liver of Mothers

	FA concentrations (mg/m^3)		
Organ	0.012	1.0	Control
Total body (gm)	6.0*	6.3†	5.6
Organ (mg/10 gm body weight)			
Thymus	25.1	31.7*	26.0
Heart	61.5	64.5	61.4
Lung	230.2*	223.2	287.1
Liver	557.9‡	550.8†	587.7
Adrenals	4.2*	3.8‡	3.2
Kidney	53.4	55.7†	51.4

	Ascorbic acid concentrations (mg %)					
	0.012		1.0		Control	
Organ	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*
Whole fetus (gm)	14.4	0.2*	14.3	0.7†	19.0	1.1
Placenta (mg/10 gm body weight)	6.8	0.7	6.4	0.7	9.7	1.4
Maternal liver (mg/10 gm body weight)	18.1	2.1†	16.8	1.1‡	20.6	0.8
Fetal liver (mg/10 gm body weight)	20.1	1.7‡	14.8	0.7	15.8	0.4

*$p < .001$.
†$p < .01$.
‡$p < .05$.

Table 4.—Activity of Enzymes of the Subcellular Organelles and Concentration of *N*-Acetyl-Neuraminic Acid in the Liver of Fetuses and Various Organs and Blood Serum of Pregnant Females in the Presence of an Embryotoxic Effect of Formaldehyde

Organ	Group of animals	Malate dehydrogenase		Glutamate dehydrogenase		Succinate dehydrogenase		ATPase		β-glucuronidase		Inosine diphosphatase		*N*-acetyl neuraminic acid	
		$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	mg	%
Blood serum	Control	1.78	0.64	0.35	0.04					4.84	0.16	0.07	0.01	141.0	4.0
	Experimental	1.35	0.19	0.37	0.03					7.70	0.38*	0.06	0.01	168.0	6.0*
Liver of female	Control	42.30	3.30	3.10	0.3	9.70	2.27	1.57	0.08	0.96	0.08	18.73	1.35	96.0	2.0
	Experimental	29.60	1.41*	4.32	0.10*	4.68	0.72*	3.08	0.14*	0.88	0.12	10.78	0.73*	124.0	4.0*
Liver of fetus	Control	13.50	1.40	3.12	0.17	0.91	0.30	3.15	0.17	0.25	0.01	3.36	0.22	154.0	8.0
	Experimental	10.60	0.54*	4.53	0.12*	1.80	0.30*	6.72	0.67*	0.16	0.02*	1.38	0.20*	188.0	6.0*
Placenta	Control	11.70	0.57	0.27	0.05	1.02	0.15	3.56	0.12	0.23	0.02	4.07	0.15	153.0	5.0
	Experimental	8.40	1.00*	0.72	0.14*	0.52	0.12*	7.74	0.52*	0.23	0.02	2.35	0.15*	154.0	3.0

Notes: The activity of all the enzymes is given in μM/min · gm, and only H^+-adenosine triphosphatase (ATPase) is given in μM/hr · mg of protein. $\bar{x}$ = mean, and *SD* = standard deviation.
*$p < .05$.

21st day of life; (d) time of appearance of fur; (e) detachment of *concha auriculae*; (f) time of eye opening; (g) body mass; and (f) organ (i.e., liver, lung, heart, spleen, adrenal gland, and thymus) mass. Organ structural indicators were analyzed at the following ages: 2 wk (i.e., newborn baby rats), 2 mo (males and females), and 4 mo (males) for liver, kidney, lung, and lung mononuclear macrophage system in bronchoalveolar lavages (BALs). Hepatocyte alteration index (HAI), ploidy, reticuloendothelial system, lymphoid macrophage infiltration, micronecrotic loci, and extramedullary hematopoiesis centers were assessed. Lung capacity was determined morphometrically. The kidney was examined for normal, atrophic, and hypertrophied glomeruli.

The liver damage included a decrease in HAI, a retention of extramedullary hemopoiesis, an increase of ploidy (up to 16n), and micronecrotic loci. In addition, there was lymphoid histiocyte infiltration and fibrosis of blood vessels. The alterations were greater in male (i.e., 2–3 times greater than controls) than in female rats. Kidneys showed atrophic glomeruli, with a proportional decrease in normal glomeruli. The lungs had a decrease in alveolar macrophages in BAL.

The mitochondrial enzyme SDH was decreased in perivascular hepatocytes at birth, at 2 wk of age, and 2–4 mo following treatment. The SDH activity was also decreased in the kidneys (tubule and glomeruli) at 2 mo and 4 mo of age. Acid phosphatase activity decreased in the kidney at 2 wk and at 2 mo of age, and it rebounded in males at 4 mo of age; this rebound occurred despite atrophic glomeruli being increased at 4 mo of age. The activity of lactate dehydrogenase (LDH) was decreased at 4 mo of age in the liver and alveolocytes (BAL cells).

The assessment of embryotoxicity according to generally accepted indices of the development of offspring is not adequate for the evaluation of the effect of FA on fetuses. A more detailed structural and functional study of the offspring during the postnatal period is required.

Embryotoxic effect of FA at Russian maximum allowable concentration (MAC). The embryotoxicity of FA and gasoline was compared with controls in the offspring of 137 female white rats and their 853 embryos.[44] Female rats that weighed 180–200 gm were mated and were exposed for 4 hr/day to FA on days 1–19 of pregnancy at the MAC (i.e., 0.5 mg/m^3) in special chambers. Gasoline at 3 times the MAC (i.e., 300 mg/m^3) was also introduced in the same manner in a second group of pregnant females. Control pregnant rats were handled in the same manner, but they were not exposed to FA or to gasoline.

No significant effects were observed on corpus lutea, embryo lost before and after implantation, implanted embryos, and length of humerus and embryos. Congenital defects included cryptochordism (20.8 ± 7.6% vs. 1.2 ± 1.2%; $p < .05$), delay in ossification of hyoid bone, delay in eruption of upper and lower incisors (days 14–15 vs. 12 of life; $p < .01$), and decrease in body weight (days 3, 9, 10, and 17 of life, $p < .05$). Analysis of blood (ABS) revealed significant hypercapnia, with changes in partial pressure of carbon dioxide (pCO_2) and partial pressure of oxygen (pO_2) (Table 5). This attests to a compensated CO_2 acidosis in the mothers and embryos. (It should be noted that the authors did not state whether the blood was venous or arterial.)

Open-field tests demonstrated an increase in motor activity (i.e., numbers of squares visited), increases in frequency of standing, and increases in the appearance of emotion (i.e., defecation and urination [$p < .01$]) in 40-day-old rats (Table 6). Motor hyperactivity was also present. In sexually mature rats, there was an increase in search activity on days 2–5 of training. The rate of appearance of motor reflexes did not differ between exposed and control rats.

Effects of FA on prenatal development of rats with induced trace-element disorder. The effect of FA and gasoline inhalation on prenatal development of rats with induced iron deficiency was reported.[45] The authors stated that the rationale behind this study was the fact that the frequency of anemia increases by a factor of 2–3 in women who come in contact with various

Table 5.—Indices of Gas Composition and pH of the Blood of Females and Offspring Exposed to Vapors of Formaldehyde and Gasoline during Pregnancy

	Exposure					
	Formaldehyde		Gasoline		Control	
Index	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*
pH						
Females	7.21	0.02	7.29	0.02	7.32	0.01
Embryos	6.90	0.03	7.03	0.03	6.98	0.02
pCO_2 (mm Hg)						
Females	54.10	2.95*	41.00	1.07	42.91	1.66
Embryos	130.48	5.55*	86.53	4.01†	100.91	3.60
pO_2 (mm Hg)						
Females	67.90	5.62	45.75	1.98	51.84	3.37
Embryos	1.23	0.49†	1.30	0.43†	8.16	2.25

Notes: $\bar{x}$ = mean, *SD* = standard deviation, pCO_2 = partial pressure of carbon dioxide, and pO_2 = partial pressure of oxygen.
*Differences from control are reliable: $p < .01$.
†Differences from control are reliable: $p < .05$.

Table 6.—Indices of "Open-Field" Behavior of Young Offspring after Prenatal Exposure to Formaldehyde and Gasoline at Different Testing Times

	Exposure					
	Formaldehyde		Gasoline		Control	
Index	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*
No. of squares						
Day 1	85	6	105	6*	76	7
Day 2	45	6*	33	3†	21	4
Day 3	57	4*	23	2†	13	2
No. of instances standing						
Day 1	9.4	0.7	15.2	0.9*	10.8	1.0
Day 2	5.0	0.7†	2.7	0.3	2.4	0.5
Day 3	4.3	0.6*	2.6	0.4*	1.1	0.3
No. of defecations and urinations						
Day 1	7.5	0.8*	6.7	0.8*	3.4	0.8
Day 2	6.8	0.8†	6.3	0.6	4.3	0.8
Day 3	7.4	0.7*	5.6	0.5	4.4	0.8
No. of washing movements						
Day 1	3.1	0.3	1.5	0.2*	2.9	0.5
Day 2	1.4	0.2	0.2	0.1*	1.6	0.4
Day 3	1.0	0.2	0.3	0.1†	0.8	0.2
Latent time (sec)						
Day 1	3.6	0.4	3.3	0.4	3.8	0.7
Day 2	3.9	0.6	0.5	0.2	2.5	1.8
Day 3	1.1	0.3†	0.7	0.2	0.2	0.2

Notes: $\bar{x}$ = mean, and *SD* = standard deviation.
*Differences from control are reliable: $p < .01$.
†Differences from control are reliable: $p < .05$.

industrial pollutants in Russia, and iron-deficiency anemia is a factor in the risk of prenatal pathology.

Pregnant white rats ($N = 254$) that had body weights of 180–200 gm (2,511 embryos) were investigated following inhalation of FA (0.5 mg/m^3) and gasoline (300 mg/m^3) in special chambers in which chemicals were administered for 4 hr/day during the 1st–9th day of pregnancy. The concentrations were controlled by the weight method. Iron deficiency was induced by intraperitoneal injection of bipyridyl (i.e., chelating agent in 25% ethanol) at the threshold embryotoxic doses (50 and 40 mg/kg, respectively) in 0.1 ml on days 12, 13, and 14 of pregnancy. The control animals were injected with ethanol. The prenatal effect of the xenobiotics was determined by morphological and biochemical methods. Statistical analysis was performed by Silcoxon-

Mann-Whitney nonparametric tests. On the 20th day of pregnancy, the females were killed by cervical dislocation. The number of corpora lutea, implantation sites, and live and reabsorbed embryos were counted. The pathology of internal organs and skeleton in embryos was recorded. The average number of metacarpal and metatarsal bone centers per limb was calculated. The acid-base state of the blood of 20-day-old embryos and of the pregnant females was measured on the "Radiometer" blood microanalyzer (Denmark).

Formaldehyde caused an increase (21%) in cryptochordism. Bipyridyl caused cleft lip and palate, adhesion, and reduction of cartilage of the sacrum and tail in a small number of animals (Table 7). Inhalation of FA with bipyridyl administration on days 12–14 of pregnancy increased anomalies over controls. There was a significant increase in embryo mortality. In addition, increases in anomalies were observed for cryptochordism (26.7%), syndactyly (0.4%), adhesion of breastbone (1.2%) and tail (6.0%), and phocomelia (1.3%). The overall frequency of developmental defects, with combined effects of FA and bipyridyl, increased more noticeably (i.e., up to 13.8%) than did the separate effect of FA (6.1%; $p < .01$) and bipyridyl (6.6%; $p < .02$).

The pH of blood in the mothers and embryos was significantly increased by bipyridyl and was decreased by FA and FA + bipyridyl. The pCO_2 in females was increased by FA exposure, whereas bipyridyl and FA + bipyridyl decreased it. In embryos, there was an increase in the pCO_2 by FA exposure and by bipyridyl exposure, whereas the combination of the two exposures decreased this parameter. Changes in the pO_2 were also found. In general, pO_2 increased in both females and embryos.

A significant decrease was observed in the base reserves of the blood true carbonates and total CO_2 in the same embryos exposed to FA + bipyridyl compared with controls, the FA exposure group, and the bipyridyl group. Moreover, there was a significant build-up of metabolic acid products in embryos in response to the combined exposure compared with controls, the FA exposure group, and the bipyridyl group.

Discussion and Analysis of the Articles

The C14FA was distributed to all organs in the adult, in the placenta, and in the fetus (Table 1)—a result that was similar to that reported in male F344 rats, guinea

Table 7.—Embryonic and Teratogenic Effects of Gasoline and Formaldehyde against a Background of Induced Iron Trace-Element Disorder in Female Rats as of Day 12 of Pregnancy

Index	Control	Gasoline	Formaldehyde	Bipyridyl	Bipyridyl + gasoline	Bipyridyl + formaldehyde
No. of pregnant females	29	30	29	18	18	28
Post-implantation mortality	(4.8 ± 1.3)†	(10.1 ± 3.2)†	(6.2 ± 1.9)†	(12.6 ± 5.5)†	(22.7 ± 7.2)†	(23.1 ± 5.9)†
Anomalies* (no. of rats)						
Harelip	0	0	0	1 (2.8 ± 2.8)†	2 (5.2 ± 3.6)†	2 (5.2 ± 3.6)†
Cleft palate	0	0	0	1 (2.8 ± 3.6)†	2 (5.2 ± 3.6)†	2 (5.2 ± 3.6)†
Hydrophenosis	1 (0.5 ± 0.5)†	3 (2.9 ± 1.9)†	3 (5.1 ± 3.0)†	0	0	0
Cryptochordism	0	0	7 (20.8 ± 2.6)†	5 (8.8 ± 4.2)†	6 (13.5 ± 5.5)†	14 (26.7 ± 6.1)†
Hydrocephaly	0	0	0	0	0	0
Oligodactylia (fore limbs)	0	0	0	0	0	0
Phocomelia (rear limbs)	0	0	0	0	0	1 (1.3 ± 1.3)†
Adhesions (no. of rats)						
Breast bone	0	0	0	0	0	2 (1.3 ± 0.8)†
Digits	0	0	0	0	0	1 (0.4 ± 0.4)†
Sacral cartilages	0	0	0	1 (1.5 ± 1.5)†	3 (11.7 ± 7.3)†	0
Tail cartilages	0	0	0	2 (0.7 ± 0.7)†	0	5 (6.0 ± 2.7)†
Partial reduction of tail cartilages	0	0	0	0	8 (33.7 ± 10.1)†	5 (9.0 ± 4.6)†
No. of embryos	221	235	230	196	166	181
Anomalies‡	0	4 (1.7 ± 0.8)†	14 (6.1 ± 1.6)†	13 (6.6 ± 2.8)†	21 (12.7 ± 2.6)†	39 (13.8 ± 2.1)†

*Frequency of anomalies per litter.
†Percentage (mean ± standard deviation) appears within parentheses.
‡Frequency of anomalies for total embryos.

pigs, and monkeys.[46,47] The major difference is that the Japanese demonstrated the incorporation of FA and its metabolites into the placenta and fetus. The quantity of radioactivity remaining in maternal and fetal tissues at 48 hr was 26.9% of the administered dose. The DNA fraction contained 20% and 50% of total incorporated radioactivity in the maternal and fetal liver at 6 and 24 hr, respectively, compared with the acid-insoluble fraction (Fig. 1). Of primary interest is that the incorporated radioactivity persisted longer in the fetal liver and in brain of the mothers. Also, given that FA is a precursor of several biological compounds, it would have been of prime interest if the investigators had determined what fraction resulted from either metabolic incorporation or from chemical reactivity of FA (e.g., crosslinks, adduction, methylation) with biological molecules (e.g., DNA, proteins, polypeptide, amino acids [AAs]).

Formaldehyde undergoes addition (adducts and alkylation) and condensation (methene bridges) reactions with proteins and AAs,[48] as well as with nucleic acids and nucleosides/tides.[49] Formaldehyde is a mutagen, a crosslinking agent, and an immunogen.[49–51] Free FA concentrations in the blood are 2.24 ± 0.07 μg/gm in rats, 1.84 ± 0.15 μg/gm in Rhesus monkeys, and 2.61 ± 0.14 μg/gm in humans; FA concentrations did not change following either acute or subchronic inhalation of FA.[52,53] Thus, it appears that additional information is required about addition and condensation products of AAs, polypeptides, and nucleosides (among others) of the blood generated by FA exposure. An increase of *N*-methyl AAs would produce endogenous FA, which may have a significant role in mitotic and apoptosis processes. Generators of FA are responsible for FA formation in tumors, and they impair liver antioxidant mechanisms and functional integrity of mitochondria.[54–62]

Formaldehyde adversely affected zygotes/embryos and bone marrow cells (Tables 2 and 3). The embryos showed cytological injury and a high rate of mortality, whereas bone marrow cells had increased rates of chromosome aberrations and aneuploidy. Similar observations on chromosomes of peripheral lymphocytes have been reported for anatomy and mortuary students.[63–65] Classroom exposure to FA at 1.5–3.17 mg/m^3 was associated with an increased frequency of sister chromatid exchanges, aberrations, and micronuclei. Formaldehyde concentrations of less than 1 mg/m^3 had no effect on lymphocyte chromosomes, but they caused micronuclei in nasal and oral exfoliative cells, as well as changes in lymphocyte subsets (increase in CD19 and decreases in CD4, CD5, and the H/S ratio).[66,67]

Additional research is needed about the effect of FA on embryos. Formaldehyde is an alkylating agent. Treatment of C3H transplacentally with *N*-ethyl-*N*-nitrosourea (an alkylating agent) has caused primordial germ cell mutations.[68] In addition, treatment of female mice within hours after mating with ethyl methanesulfanate, ethyl nitrosourea, and ethylene oxide resulted in fetal deaths and malformations.[69–72] Thus, further investigation into the zygote/embryonic effects of FA should follow the protocols established for other alkylating agents, with attention to the role of potential methyl donors (e.g., *N*-methyl AAs).

Exposure to FA throughout gestation caused decreased DNA and RNA concentrations, increased weights of bodies and organs (i.e., thymus, heart, kidneys, and adrenals), and decreased weights of lung and liver (Table 3). Microscopy and histochemical observations revealed the following additional abnormalities: involution of lymphoid tissue, numerous extra-medullary hemopoietic centers, decreased glycogen content in the myocardium and liver, and decreased AA content in the whole fetus and in fetal and maternal liver. AA is an antioxidant, produced from glucuronate via the uronic acid pathway, which also is the intermediary route for synthesis of pentoses. The decreased AA content may have resulted from either the utilization of AAs as an antioxidant or by interference (inhibition?) of the uronic pathway. The meaning of the decreased DNA and the increased RNA contents of the organs is difficult to determine. However, treatment of adult male rats by FA injection reportedly decreased the DNA content of testis and prostate and decreased the protein content of the prostate and epididymis.[73]

Cytopathology of organs and alterations of mitochondria, ER, and lysosome enzymatic activity were observed in fetuses following FA inhalation (Table 4). Organ cytopathology included increased ploidy, micronecrotic loci, extramedullary hematopoietic centers, and degeneration of kidney glomeruli. There were concomitant changes in enzymatic activity, as follows: mitochondria (MDH, SDH, and LDH decreased, whereas GDH increased); ER and lysosomes (adenosine triphosphatase increased, whereas inosine diphosphatase and β-glucuronidase decreased). The impairment lasted in the organs up to 4 mo of age. In addition, *N*-acetylneuraminic concentration increased in maternal and fetal tissues. The changes in enzymatic activity and *N*-acetyleneuraminic acid correlated with increased fetal mortality. Finally, the development of postnatal behavior was also adversely affected (Table 6).

Formaldehyde has effects on mitochondrial enzymes, glutathione concentrations, and bile production in the liver of many species, including humans.[74] Formaldehyde inhibits the uptake of phosphate by mitochondria[75,76] and causes the release of glutamic-pyruvic transaminase, SDH, glutathione, and malondialdehyde into the perfusate of isolated livers.[77] Intraperitoneal injection results in a 2-fold increase in bile, and there is a significant decrease in glutathione in the liver, lungs, and brain.[78] An electron microscopic investigation of the perfused isolated livers revealed destruction of the mitochondria (i.e., ruptured membranes and loss of the cristae) and some damage to the ER.[77] The protection of the liver from FA toxicity appears to be dependent on glutathione by formation of the adduct *S*-hydroxymethylglutathione.[79] Thus, the observed effect of FA on mitochondrial and ER functions during embryo/fetal development is also demonstrable in the adult liver.

Formaldehyde caused preimplantation and prenatal and postnatal abnormalities. The prenatal effects were demonstrable as anomalies and aberrancies in blood

buffering capacity with metabolic (formate?) acidosis. The major anomalies were an increased frequency of cryptochordism; a decrease/delay in ossification centers of the hyoid, metacarpus, and metatarsal bones; delay in eruption of incisors; and a decrease in body weight. Blood pH decreased in the fetus, whereas the pCO_2 (hypercapnia) increased in the fetus and in the mother. The true bicarbonates and CO_2 were unaffected by FA alone, but they increased with iron deficiency in the fetus and in the mother. The presence of iron-induced deficiency augmented these abnormalities, along with increased embryo mortality. The postnatal effect of FA was tested by maze performance. Open-field tests demonstrated an increase in motor activity, an increase in standing, and appearance of emotion. In sexually mature rats, there was an increase in search activity.

Formaldehyde is metabolized to formate. Alcohols, particularly methanol and ethanol, are metabolized to formate and lactate via an aldehyde. The toxicity of alcohols and formalin in humans and animals includes metabolic acidosis.[80–82] Alcohol toxicity generates free radicals, causes an increase in malondialdehyde, and induces lipid peroxidation, thus resulting in DNA single-strand breaks.[83–87] Formaldehyde and alcohols likely affect embryos and the fetus via mitochondrial damage. Ethanol and environmental agents trigger apoptotic neurodegeneration in the developing brain.[88,89] Oxygen stress, such as that caused by free-radical generation, is associated with apoptotic cell death and fragmentation of mitochondrial genome.[90–92] Moreover, FA via formaldehyde generators (e.g., alkylating agents) initiates apoptosis.[93–95] Mitochondria are the suicide organelles, and they control apoptosis.[96–99] Thus, subtle birth defects (e.g., autism, low birth weight, fetal alcohol syndrome) are probably best understood by investigation of in utero oxidative stress and mitochondrial damage, rather than by standard FA teratogenic research.[1–4,6]

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