The evaluation of developmental toxicity of chemicals exposed occupationally using whole embryo culture

SHU-FEN ZHAO*, XIU-CHI ZHANG, LAN-FEN ZHANG, SHU-SEN ZHOU, FAN ZHANG, QIU-FENG WANG, YU-LING WANG and YU-SHU BAO

Department of Occupational Health, School of Public Health, Beijing Medical University, Beijing, P.R. China

ABSTRACT The purpose of this study was to employ the whole embryo culture (WEC) system to evaluate the developmental toxicity of industrial chemicals. Five chemicals including lead, cadmium, vinyl chloride, 1,2-dichloroethan, and carbon disulphide were tested in our laboratory both *in vitro* and *in vivo* (except lead). *In vitro* studies showed that cadmium and lead were teratogenic in the rat; whilst carbon disulphide, 1,2-dichloroethan and vinyl chloride mainly induced embryo growth retardation. The *in vitro* effects on development of the five industrial chemicals were similar to the effects *in vivo*. The *in vitro* effects were studied by three different exposure routes, direct exposure – chemicals added to the culture medium; indirect exposure – serum prepared from treated rats then used as culture medium, and pre-exposure – embryos treated maternally then explanted into control (untreated) culture medium. Comparing these three different exposure routes suggests that the last exposure route is the most effective when using WEC to evaluate developmental toxicity of industrial chemicals. The effects on embryo development of culturing in sera prepared from subjects occupationally exposed to antineoplastic drugs (ADs) was also tested by the WEC system. Embryos were cultured with human serum that was thought to contain ADs or ADs' metabolic materials (serum taken from nurses routinely handling ADs), to evaluate the effects of ADs on embryo development. Embryos (9.5-day) cultured with serum from 11 female nurses who had been handling ADs for 2-17 years in the oncology department all survived, but showed slight growth retardation. Embryos cultured with serum from 30 healthy and unexposed people served as controls and embryo development in their serum was normal.

KEY WORDS: *whole embryo culture, industrial chemicals, developmental toxicity, reproductive toxicity, teratogenicity*

Introduction

The significance of environmental agents in the etiology of congenital malformations has been recognized for many years. Large numbers of chemicals are emitted into the environment every year, and industrial chemicals are an important pollution source. Our scientists have been devoting much attention to studying the developmental toxicity of industrial chemicals, and various teratogenicity tests have been designed for testing the developmental toxicity of drugs and other agents. The traditional teratogenicity test using the standard whole animal or segment II teratogenicity test is impracticable for the screening of the increasingly large number of chemicals introduced into the environment each year. The whole embryo culture (WEC) system was developed by New and his colleagues in Cambridge (New, 1978), with advantages including rapidity, economics, and precise control of experimental conditions. This technique has been utilized for teratogen screening and the study of teratogenic mechanisms.

The teratogenic effects of cadmium (Cd) have been described in a number of *in vivo* and *in vitro* studies (Klein *et al.*, 1980; Warner *et al.*, 1984). Cadmium-induced malformations encompass a broad spectrum of abnormalities including neural tube, limb, craniofacial and skeletal defects. In one series of experiments, serum was prepared from rats at intervals after injection of cadmium chloride, and embryos showed various abnormalities and growth retardation.

The embryo toxicity and teratogenicity of lead (Pb) in animals have been reported by McClain and Becker (1975), Kimmel *et al.*, (1980) and Beaudoin and Fisfer (1981), and embryo growth retardation and malformations were found. Carbon disulphide (CS_2) is a volatile solvent; its most important use in industry is in the production of viscose rayon fibres. It is also used, to some extent, as a solvent in various industrial processes. The teratogenicity of

Abbreviations used in this paper: DCE, 1,2-dichloroethan; VCM, vinyl chloride monomer; WEC, whole embryo culture.

***Address for reprints:** Department of Occupational Health, School of Public Health, Beijing Medical University, Beijing 100083, P.R. China. FAX: 86.10.64260503.

0214-6282/97/$05.00

Printed in Spain

TABLE 1

THE *IN VIVO* EFFECTS OF CADMIUM ON EMBRYONIC AND FETAL DEVELOPMENT IN RATS

Parameters	Cadmium doses			
	Control	2 mg/kg	4 mg/kg	8 mg/kg
No. of litters	13	16	15	14
No. of corpora lutea	15.5	13.6	14.4	15
No. of implants#	14.3±0.44	11.6±0.76	12.9±0.72	13.7±0.61
No. of live fetuses#	13.4±0.54	9.7±1.03*	9.3±1.42*	7.3±1.63**
Fetal death (%)#	6.5±1.8	15.3±2.7**	24.0±3.2**	39.6±3.8**
Fetal body weight (g)				
male#	4.0±0.1	4.2±0.1	4.2±0.1	2.9±0.3**
female#	3.8±0.1	3.9±0.1	3.8±0.1	2.9±0.3**
Fetal crown-rump Length (mm)				
male#	36.0±0.3	36.2±0.5	35.9±0.6	31.4±1.5**
female #	35.0±0.3	35.5±0.4	34.7±0.6	31.0±1.6**
External malformations	0/174(0)	0/155 (0)	3/139 (2.2)	5/102 (4.9)*
	0/61 (0)	2/53 (3.8)	2/48 (4.2)	4/35 (11.4)*

#Mean±SE; *p<0.05; **p<0.01; ***p<0.001. Figures in parentheses are percentages.

CS_2 is still a controversial issue. It has been reported in animals only by Tabacova (1976), Tabacova *et al.* (1978) and a few other authors (Bariljak *et al.*, 1975). A significant increase in pre-implantation loss and fetuses with external malformations was observed. 1,2-dichloroethan (DCE) is a volatile liquid, and is principally used in the synthesis of vinyl chloride. Vozovaya (1976) exposed female rats to 15±3 mg DCE/m^3, for 4 h daily, 6 days/week, for 4 months prior to mating, after which exposure was continued. Pre-implantation losses were found to be 5 times greater in treated animals than in the controls, though no fetal abnormalities were reported. DCE was also administered to female albino rats at a concentration of 57±10 mg/m^3 in air, for 4 h daily, 6 days/week, for 6 and 9 months. The rats were then mated and exposure continued. A reduction in fertility was observed, the weight of newborn rats was reduced, and prenatal mortality was increased (Vozovaya and Malyarova, 1975). No abnormalities were observed. Vinyl chloride monomer(VCM) is a carcinogen and mutagen, however its teratogenicity has not been established in animals. Using inhalation doses up to 2500 ppm VCM during pregnancy no malformations were found (John *et al.*, 1977; Ungvary, 1978).

Many ADs used in cancer treatment have been found to be mutagenic, carcinogenic and teratogenic in laboratory animals. Moreover, some ADs have been shown to be teratogenic in pregnant women being treated with ADs (Sorsa *et al.*, 1985; McDiarmid and Egan, 1988; Skov *et al.*, 1992). ADs have also been reported to increase the number of chromosome aberrations, sister chromatid exchanges, point mutations, micronuclei in lymphocytes, and to increase urinary mutagenicity and thioether excretion in nurses handling them (Oestreicher *et al.*, 1990; Sardas *et al.*, 1991). Recently some epidemiological studies have shown that nurses handling antineoplastic drugs face potential reproductive hazards (Hemminki *et al.*, 1985; Selevan *et al.*, 1985; Stücker *et al.*, 1990; Zhao *et al.*, 1993).

For a long time, many authors have suggested that the WEC system could be used as a screening system for teratogens and studying of teratogenic mechanisms, however, the application of the WEC system to the screening of industrial chemicals has been very limited.

There are about 56 million working women in China. They may be exposed to occupational hazards, especially industrial chemicals, whilst participating in occupational activities. An attempt has been made to expand the WEC system to screen industrial teratogens and to evaluate the developmental toxicity of industrial chemicals.

The purpose of this study was to establish the WEC system, employ it to detect the developmental toxicity of industrial chemicals, and to explore whether cultured rat embryos in human serum could be used to evaluate the teratogenic activity of serum taken

TABLE 2

EVALUATION OF EMBRYO DEVELOPMENT FOR EMBRYOS DIRECTLY EXPOSED TO CADMIUM

Group	Serum cadmium μg/ml	Yolk sac diameter (mm)#	Crown-rump length (mm)#	Head length (mm)#	No. of somites#	Protein μg/embryo#	Morphologic score#
Control N= 14	0	4.41±0.2	3.46±0.2	1.74±0.1	26.1±0.1	278.9±16.7	41.5±0.3
Cd exposure N= 10	3.2	3.40±0.23**	2.43±0.2**	1.26±0.1**	18.5±2.3**	128.1±27.0***	30.4±3.1***

*p<0.05; **p<0.01; ***p<0.001; #Mean±SE.

TABLE 3

TYPES OF MALFORMATIONS IN EMBRYOS DIRECTLY EXPOSED TO CADMIUM

Group	No. normal embryos		Complete rotation		Closed posterior neuropore		Somite disturbance		Pericardical edema		Anophthalmia	
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
Control N= 14	14	(100)	14	(100)	14	(100)	0	(0)	0	(0)	0	(0)
Cd exposure N= 10	1	(10**)	1	(10**)	1	(10)	3	(30)	1	(10)	2	(20)

**p<0.01. Figures in parentheses are percentages.

TABLE 4

TYPES OF MALFORMATIONS IN EMBRYOS PRE-EXPOSED TO CADMIUM

Group	Abnormal embryos	Axial rotation	Open neuropores	Pericardial edema (%)	Anophthalmia
Control N= 14	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2 mg Cd/kg N=16	11 (68.8)**	5 (31.3)	8 (50.0)***	4 (25.0)	2 (18.2)
8 mg Cd/kg N=9	8 (88.9)**	6 (66.7)*	8 (88.9)***	4 (44.4)**	6 (66.7)***

*p<0.05; **p<0.01; ***p<0.001. Figures in parentheses are percentages.

from nurses exposed occupationally to antineoplastic drugs. Five industrial chemicals were tested: 2 potential teratogens in animals (lead, cadmium), 2 compounds reported to have no teratogenic effect on *in utero* development but showing developmental toxicity (Vinyl chloride and 1,2-dichloroethan), and one suspected teratogen (carbon disulphide). We have compared the responses *in vivo* (teratogenicity test) and *in vitro* (WEC system) for 5 compounds (except lead) at our laboratory. Some industrial chemicals are in the form of vapour or aerosol in the air of workplaces, and they enter the maternal body through the respiratory tract, then affect embryos. Therefore the actions of industrial chemicals on embryos in culture were examined with various exposure routes, especially the effects on subsequent embryo development in culture of exposing pregnant rats to these chemicals. The effects of chemicals at different concentrations (or doses), especially low levels close to human exposure levels, on the development of embryos were observed. Using the WEC system with human serum to evaluate the effects on embryo development of occupational exposure to ADs was also explored.

Results

The effects of cadmium (Cd) on embryonic development of rat embryos in vitro and in vivo

In vivo effects of cadmium on growth and development of embryos: pregnant rats on day 7, 9, 11 of gestation were injected subcutaneously with $CdCl_2$ at doses of 0, 2, 4 and 8 mg/kg. On day 20 of gestation, all pregnant rats were killed. The results showed that the incidence of fetal death in treated groups was significantly higher than that of the controls. The fetal body weights and crown-rump lengths were also significantly lower than those of controls, and the incidence of external or internal malformations was significantly increased in the high-level exposure group, compared with controls. The types of malformation included gastroschisis, cleft palate, polydactyly, small lungs, and nephredema (Table 1) (Zhou *et al.*, 1990).

In vitro effects of cadmium on growth and development of embryos: effects of cadmium by different exposure routes were studied on cultured embryos.

Direct exposure

Cadmium was added directly to the culture medium (Cd in medium 3.2 μg/ml). 9.5-day rat embryos were treated for 30 min with cadmium; all survived a 48-hour culture period (absence of an embryonic heart beat was used as the criterion of death), but showed high malformation rates and exhibited a variety of abnormalities (Tables 2,3). These responses were similar to those observed in the pre-exposure route group (see below), except that exencephaly was not observed in the direct exposure group.

Indirect exposure

Effect on embryo development of sera after a single administration of cadmium: an attempt was made to evaluate the teratogenic activity of sera from rats exposed to cadmium. 9.5-day rat embryos were cultured for 48 h in serum taken at various times (1, 12, 24 h

TABLE 5

EVALUATION OF EMBRYO DEVELOPMENT FOR EMBRYOS PRE-EXPOSED TO CADMIUM

Group	No. embryos	Yolk sac diameter (mm)#	Crown-rump length (mm)#	Head length (mm)#	No. of somites#	Protein/ μg/embryo#	Morphologic score#
Control	14	4.41±0.2	3.46±0.20	1.74±0.1	26.1±0.1	278.9±16.7	41.5±0.3
2 mg Cd/kg	16	4.21±0.9	2.85±0.18 *	1.46±0.1	20.3±1.6 **	203.9±20.5 **	35.3±2.1 **
8 mg Cd/kg	9	3.71±0.1	3.01±0.26	1.36±0.1 *	10.4±0.1 ***	–	21.6±3.0 ***

#Mean±SE; *p<0.05; **p<0.01; ***p<0.001.

TABLE 6

EVALUATION OF EMBRYO DEVELOPMENT FOR EMBRYOS DIRECTLY EXPOSED TO LEAD (μg/ml)

Group	No. embryos	Yolk sac diameter (mm)#	Crown-rump length(mm)#	Head length (mm)#	No. of somites#	Protein/ μg/embryo#	DNA μg/embryo#
Control	20	4.89±0.12	3.25±0.06	1.79±0.06	26.00±0.40	240.98±7.83	25.07±0.87
30.68	20	4.43±0.09 **	3.31±0.09	1.71±0.06	22.00±0.78 **	176.32±5.06	17.97±1.00 **
62.88	20	3.90±0.16 **	2.75±0.16 **	1.38±0.09 **	19.50±0.41 **	98.43±7.43 **	12.25±0.66 **
88.64	20	3.21±0.10 **	2.29±0.09 **	1.03±0.06 **	15.25±1.14 **	52.20±4.64 **	8.83±0.83 **

#Mean±SE; *p<0.05; **p<0.01; ***p<0.001.

TABLE 7

EVALUATION OF EMBRYO DEVELOPMENT FOR EMBRYOS PRE-EXPOSED TO LEAD (50 mg/kg)

Group	No. embryos	Yolk sac diameter (mm)#	Crown-rump length (mm)#	Head length (mm)#	No. of somites#	Protein/ μg/embryo#	DNA μg/embryo#
Control	20	4.96±0.03	3.56±0.05	1.95±0.03	29.2±0.48	322.4±9.17	32.76±1.19
Pb exposure	20	4.22±0.03	3.40±0.05	1.87±0.04	24.5±0.65*	221.8±12.81*	20.05±1.08**

#Mean±SE; *p<0.05; **p<0.01.

TABLE 8

EVALUATION OF EMBRYO DEVELOPMENT FOR EMBRYOS PRE-EXPOSED TO CS_2

Group	No. embryos	Yolk sac diameter (mm)#	Crown-rump length (mm)#	Head length (mm)#	No. of somites#	Protein/ μg/embryo#	Morphologic score#
Control	17	4.35±0.09	3.18±0.08	1.48±0.06	23.6±0.3	219.3±19.8	42.8±0.5
100 mg/m^3	10	3.97±0.17**	2.69±0.11***	1.21±0.09*	20.3±0.5*	78.3±12.8***	37.1±1.3***

#Mean±SE; *p<0.05; **p<0.01; ***p<0.001.

after dosing) from treated rats (Cd 2 mg/kg). The results demonstrated that embryo growth was affected and the effect was different in serum from animals treated with cadmium at various times. The cadmium in serum was measured; concentrations were different at different times after exposure, and were 1.98 mg/ml, 0.14 mg/ml, 0.08 mg/ml, 0.06 mg/ml at 1, 4, 8, 12 h, respectively. Serum cadmium reached the highest levels at about 1 h after injection and thereafter declined rapidly within 4 h, and was not detectable 24 h after administration. All embryos cultured for 48 h in serum taken 1 h after cadmium administration failed to thrive. Embryos in serum taken 8 or 12 h after cadmium administration survived, but were smaller and contained less protein than controls. In serum, from rats treated 24 h prior to serum preparation, embryos appeared morphologically normal but contained reduced amounts of protein. When tissue sections were examined, two embryos exhibited abnormal eye development including anophthalmia (in two cases).

Pre-exposure

Pregnant rats were injected on day 8.5 subcutaneously with 2 mg/kg and 8 mg/kg Cd, respectively, then embryos were explanted on day 9.5 into serum taken from untreated rats and cultured for 48 h. The results showed a high malformation rate and a variety of abnormalities in embryos from treated rats (Table 4). These defects included failure to complete axial rotation, anophthalmia, open neuropores and pericardial edema. When compared to controls, development of these embryos was retarded, and the yolk sac diameters, head lengths, and crown-rump lengths were significantly decreased. Furthermore, total embryonic morphological scores were lower, and embryo protein was significantly reduced. The effects showed a dose-response relationship (Table 5).

The effects of lead (Pb) on the development of embryos in vitro

Direct exposure

9.5-day rat embryos were cultured for 48 h in medium containing final concentrations of 0, 30.68, 62.88 or 88.64 μg/ml Pb^{++}. All embryos survived a 48 hour culture period, but showed significant decreases in visceral yolk sac circulations, yolk sac diameters, crown-rump lengths, and head lengths. The development of somites, limb buds and branchial bars was inhibited, and protein and DNA synthesis were reduced. A dose-response relationship was found. Developmental abnormalities such as open neural tubes, failure of axial rotation, and malformations of brain and heart were observed (Table 6) (Zhang *et al.*, 1992).

Pre-exposure

Pregnant rats received an intraperitoneal injection of lead acetate (50 mg/kg) on the 9.5th day of gestation. After 24 h, embryos (10.5 day) were explanted into serum prepared from untreated rats, and cultured for 24 h. On the next day, all embryos had survived, but showed significant decreases in yolk sac diameter, number of somites, growth of limb buds, and protein and DNA synthesis. Malformations such as open neural tubes and failure of axial rotation were observed (Table 7) (Zhang *et al.*, 1992).

The effects of CS_2 on growth and development of embryos in vivo

In our laboratory, teratogenicity tests were made on Wistar rats and Kun Ming mice. The results showed that when pregnant rats and mice were exposed to CS_2 at concentration of 10 or 100 mg/m^3 throughout gestation, no teratogenic effects were found. However some embryo toxicity such as delayed skeletal ossification and pericardia hemorrhage of newborns was observed in the 100 mg/m^3 exposure group (Wang and Bao, 1988).

The effects of CS_2 on development of rat embryos in vitro

Pre-exposure

Pregnant rats were exposed to carbon disulphide(100 mg/m^3, 4 h/day) on day 7 and day 8 of gestation and embryos explanted from them at 9.5 days into serum taken from untreated rats. The embryos were cultured for 44 h. The results showed that in the exposure group, yolk sac diameters, crown-rump lengths, head lengths, and embryo protein contents were reduced, and somite,

limb bud and bronchial bar formation were inhibited. The incidence of failure to complete axial rotation (50%) was significantly higher than for controls (5.9%). The incidence of open posterior neuropores in exposed embryos was higher than for controls, but the difference was not statistically significant. The results suggested that the effects of carbon disulphide on cultured embryos were similar to those *in vivo;* embryonic toxicity was found but no significant teratogenicity exhibited (Table 8).

The effects of DCE on the growth and development of embryos in vivo

Rats were exposed to DCE at concentrations of 0, 24.8±0.7 and 207.6±10.2 mg/m^3, for 6 h per day from two weeks before mating and throughout gestation. On day 20 of gestation, all pregnant rats were killed by cervical dislocation. The results showed that the incidence of pre-implantation loss in the group exposed to DCE at a concentration of 207.6 mg/m^3 (31.0%) was significantly higher than in the control group (10.2%), ($p<0.05$) and the male pup weights in the exposure group (3.9 g) were significantly lower than for the control group (4.4 g), ($p<0.05$). Gross skeletal, and visceral malformations were not found (Zhao *et al.,* 1984, 1989).

The in vitro effects of DCE pre-exposure

Pregnant rats on day 7 and day 8 of gestation were exposed to DCE at concentration of 25 and 250 mg/m^3 respectively, for 4 h daily. Then embryos were explanted on day 9.5 into serum taken from untreated rats, and cultured for 44 h. The results showed that embryo development was retarded; yolk sac diameters, head lengths, crown-rump lengths, and protein contents were significantly lower in the 250 mg/m^3 DCE exposure group than in controls. The embryos in the high-exposure group appeared grossly normal, but open posterior neuropores were found (21.4% vs 5.9% in controls). No difference was found between the group exposed to 25 mg/m^3 DCE and the controls (Table 9).

The effects of VCM on growth and development of embryos in vivo

Teratogenicity tests of VCM in rats and mice were made. On day 20 of gestation, pregnant rats were killed (pregnant mice were killed on day 18 of gestation). No teratogenic effects were found in rats or mice when exposed to 5,000 ppm VCM during pregnancy. However, a slight embryo toxicity manifested as subcutaneous bleeding, or delayed ossification of the skeleton was observed in the pups of mice and rats exposed to 5,000 ppm VCM (Bao *et al.,* 1979).

In order to screen potential teratogens, another test of VCW exposure *in vivo* was undertaken. Pregnant rats were exposed to VCM by inhalation on day 7 to day 10 of gestation at concentrations of 0, 50 ppm and 500 ppm. Rats were killed on gestation day 11.5, and the embryos were observed and evaluated using a morphological scoring system (Brown and Fabro, 1981). The results showed that embryonic morphological scores, crown-rump lengths, head lengths and embryo protein contents were significantly lower than those of controls in both the exposure groups ($p<0.05$). No teratogenic effect was found (Table 10) (Zhang *et al.,* 1993).

VCM exposure in vitro

Pre-exposure: pregnant rats were exposed to VCM on day 7 and day 8 of gestation, at concentrations of 0, 50, 500 ppm respectively. Their embryos (9.5 day) were then explanted into serum prepared from untreated rats, and cultured for 48 h. The results were similar to those observed *in vivo* (Zhang *et al.,* 1993).

The effects on embryonic development of occupational exposure to Antineoplastic drugs (ADs)

Culture of rat embryos on human serum

Head-fold-stage (9.5 day) rat embryos were cultured for 48 h in human serum supplemented with glucose (3 mg/ml serum) and 10% rat serum. Comparison of embryo development in rat serum and in human serum showed that growth and development were similar in the two sera, and there was no growth retardation or abnormal development. Apart from the DNA contents of the embryos, there were no statistically significant differences between the two groups (Table 11).

9.5 day rat embryos were cultured for 48 h in serum from nurses. The results showed embryo growth retardation, reduced protein contents, and somite numbers, and branchial-bar formation was inhibited. Morphological scores of embryos were reduced in the serum of nurses occupationally exposed to ADs, compared with controls ($p<0.05$) (Table 12).

Discussion

Whilst the value of the WEC system in the study of mechanisms of normal and abnormal development may be undisputed, its potential value in screening for teratogenic agents or the evaluation of the developmental toxicity of industrial chemicals has been reported rarely. In this review, an attempt has been made to demonstrate the broad application of WEC. We have compared the effects *in vivo* and *in vitro* for 5 distinct industrial chemicals and have found that the *in vitro* effects were similar to those *in vivo*.

The teratogenic effects of cadmium have been reported previously using similar whole embryo culture techniques (Klein *et al.,* 1980; Record *et al.,* 1982). In our research, one series of experimental sera was prepared from rats at intervals after injection of cadmium chloride. Embryos treated with the sera showed various abnormalities, including failure of closure of the fore-and mid brain, and retarded growth. The effects on the embryos showed dose-response relationships which may be due to the differences in cadmium concentrations in sera taken at different times. The cadmium concentration in serum from treated rats was measured.

Kimmel *et al.* (1980) examined the effects of lead (Pb) on the growth and development of embryos. Rat were exposed to Pb-acetate for 6-7 weeks, then mated and exposed continually throughout gestation and lactation. Their offspring showed delayed vaginal opening in the 50 and 250 ppm groups, and significant growth retardation 1 to 3 weeks after exposure. McClain and Becker (1975) reported that single doses of 25-70 mg/kg of lead nitrate administered (iv) to pregnant rats on day 9 of gestation, caused a urorectocaudal syndrome of malformations and skeletal anomalies.

In our study, using the WEC system, embryos were directly exposed to Pb in the medium, or the embryos were taken from pretreated pregnant rats. The results showed that although all embryos survived for 24 h in culture, growth retardation and developmental malformations in the brain and heart were ob-

TABLE 9

EVALUATION OF EMBRYO DEVELOPMENT FOR EMBRYOS PRE-EXPOSED TO DCE

Group	No. embryos	Yolk sac diameter (mm)#	Crown-rump length (mm)#	Head length (mm)#	No. of somites#	Protein/ μg/embryo#	Morphologic score#
Control	17	4.36±0.09	3.18±0.08	1.48±0.06	23.6±0.3	219.3±19.8	42.8±0.5
25 mg/kg	12	4.96±0.22	3.31±0.10	1.60±0.05	24.2±0.4	269.5±23.9	40.0±0.4*
250 mg/kg	14	3.47±0.18***	2.48±0.11***	1.16±0.07***	20.0±0.9***	124.3±20.5**	38.4±1.1**

#Mean±SE; *p<0.05; **p<0.01; ***p<0.001.

served, and a dose-response relationship was found. Our study shows that cadmium and lead are potential teratogens.

The teratogenic effects of CS_2 in animals have been reported only by Tabacova (Tabacova, 1976; Tabacova *et al.*, 1978) and a few other authors. Tabacova demonstrated that when pregnant Albino strain rats were exposed to CS_2 at concentrations of 50, 100 and 200 mg/kg throughout gestation for 8 h per day, significant increases in preimplantation loss and externally malformed fetuses resulted.

In our laboratory, no significant teratogenic effects of CS_2 were found *in vivo* (Wang and Bao, 1988). Using the WEC system, it was found that in the exposure group, the yolk sac diameters, crown-rump lengths, head lengths, and embryo protein contents were reduced, and somite, limb bud and branchial bar formation were inhibited. The incidence of failure to complete axial rotation was significantly higher in controls. Embryos with open posterior neuropores had fewer somites (<22 somites), though the difference was not statistically significant compared with controls. The result suggested that the effects of CS_2 on embryos *in vitro* were similar to the effects *in vivo* (Zhao *et al.*, 1992). CS_2 was found to be embryotoxic, but not teratogenic.

John *et al.* (1977) exposed rodents and rabbits to different concentrations of VCM at 50 to 2500 ppm during organogenesis and found no adverse fetal effects. Ungvary (1978) exposed rats to 1500 ppm VCM during pregnancy and produced increased fetal mortality but no malformations. In this study, teratogenicity of VCM was not found either in 11.5-day embryos or in 20-day fetuses. The effects of DCE on embryonic development were tested both in our laboratory and by Vozovaya (1976) and Vozovaya and Malyarova (1975). The responses were similar; the result showed that DCE is embryotoxic but no teratogenic. Our study showed that the effects of five industrial chemicals on embryos *in vitro* were similar to those *in vivo*, and suggests that WEC may prove an effective method of evaluating the effects of such chemicals.

Some industrial chemicals are in the form of vapour or aerosol in the air of workplaces, and enter the maternal body through the respiratory tract, then affect the embryo. The effects of industrial chemicals on *in vitro* embryos were therefore examined by various exposure routes. We designed three different routes of exposure: directly adding chemicals to the culture medium, using serum from rats given industrial chemicals, and explanting embryos from treated pregnant rats into serum taken from untreated rats. The response in each case was similar. The exposure route of treating pregnant rats with chemicals and then explanting their embryos into serum taken from untreated rats provides a complete model of the "embryo/placenta/mother" unit .

In order to screen potential teratogens, we observed directly 11.5-day embryos that had been exposed *in vivo* (VCM), which provides a rapid test-method for evaluation of the development toxicity of industrial chemicals.

Using the WEC system to evaluate the developmental toxicity of industrial chemicals, some differences in embryonic response may be related to differences in exposure time, concentration (dose) and animal strains. The evaluation of the teratogenic activity of serum could be affected by the time of preparation of serum since chemical levels in serum may decline rapidly.

When we evaluate the developmental toxicity of chemicals in WEC, we should distinguish embryo growth retardation from malformation using the method reported by Steele *et al.* (1983). In our study, although incomplete axial rotation and open neuropores were found in embryos exposed to CS_2, DCE and VCM, they also had less somites. The evaluation of the effects of chemicals should be made at different concentrations (doses), especially at low levels to which humans may be exposed.

Comparing the results of this study *in vitro* and *in vivo*, it seems that the susceptibility of embryos *in vitro* is greater than *in vivo*. For example, pregnant rats exposed to Cd at concentration of 2 mg/kg, did not have malformed embryos. However, when embryos were explanted from pregnant rats injected with 2 mg/kg Cd and cultured, a high malformation rate was found.

We have also shown that head-fold-stage (9.5 day) rat embryos cultured in human serum may be used as a method of monitoring the effects on development of exposure to antineoplastic drugs.

Materials and Methods

Animals

Wistar rats were provided by the Animal Centre of Beijing Medical University. Males and virgin females were housed together overnight, and

TABLE 10

THE EFFECTS ON EMBRYO DEVELOPMENT *IN VIVO OF EXPOSING* PREGNANT RATS TO VCM

Group VCM	No. of embryos	Head length (mm)#	Protein μg/embryo#	Morphologic score#
500 ppm	18	1.92±0.04 ***	229.32±11.47***	39.9±0.4***
50 ppm	10	2.16±0.05	200.40±8.14 ***	43.9±1.0
Control	9	2.14±0.04	313.76±8.72	42.0±0.5

#Mean±SE; *p<0.05; **p<0.01; ***p<0.001.

TABLE 11

MORPHOLOGICAL FINDINGS ON 9.5-DAY RAT EMBRYOS CULTURED IN HUMAN SERUM AND IN RAT SERUM FOR 48 h

Group	Yolk sac diameter (mm)#	Crown-rump length(mm)#	Head length (mm)#	No. of somite#	Protein/ μg/embryo#	Branchial bar#	DNA μg/embryo#
Rat serum N= 31	5.87±0.07	4.21±0.08	1.85±0.03	26.10±0.20	272.93±11.88	3.0±0.0	22.88±1.03
Human serum N= 93	5.98±0.05	4.09±0.05	1.90±0.02	26.11±0.13	282.27±5.53	2.9±0.03	20.30±0.33*

#Mean±SE; *p<0.05.

TABLE 12

MORPHOLOGICAL FINDINGS ON 9.5-DAY RAT EMBRYOS CULTURED IN SERA FROM NURSES AND IN CONTROL SERA

Group	No. of embryos	Yolk sac diameter (mm)#	Crown-rump length (mm)#	Head length (mm)#	No. of somite#	Protein μg/embryo#	Branchial Bar#
Nurse serum	41	5.78±0.08	4.09±0.08	1.85±0.05	24.15±0.49**	242.37±10.29**	2.73±0.08*
Control	93	5.98±0.05	4.09±0.05	1.90±0.02	26.11±0.13	282.27±5.53	2.91±0.03

#Mean±SE; *p<0.05; **p<0.01.

the females were checked for the presence of vaginal plugs at 9:00 am. The plug day was defined as day 0 of pregnancy (0.5 day at noon).

In vivo studies

Pregnant rats were randomly assigned to experimental groups, and exposed to various doses of industrial chemicals. On day 20 (Cd, CS_2, VCM) or 11.5 (VCM) of gestation, all pregnant rats were killed by cervical dislocation (pregnant mice were killed on day 18). The uteri were removed and implantation sites, resorptions and dead fetuses were recorded. Live fetuses were sexed, weighed, and examined under a dissecting microscope for the presence of external anomalies. About half of the fetuses were fixed in 95% ethanol, cleared in 1% KOH, and stained with alizarin red S for skeletal examination. The remaining half of the fetuses from each group were fixed in Bouin's fluid and examined by microdissection for internal soft tissue abnormalities.

In vitro studies

Embryos were explanted in the morning at 9.5 or 10.5 days of gestation with Reichert's membrane torn open, but the visceral yolk sac and ectoplacental cone were left intact. The embryos were cultured at 37°C for 44-48 h in rotating 30-ml glass-stoppered bottles. The culture medium was pure rat serum centrifuged immediately after withdrawal from the donor, and heat inactivated (30 min at 56°C) before use (New, 1978). Each culture bottle contained 3 embryos in 4 ml of serum and was gassed with a mixture consisting of 5%O_2/5% CO_2/90%N_2, 20%O_2/5% CO2/75%N_2 and 40%O_2/5% CO_2/55% N_2 at different culture times (0, 24 and 36 h, respectively).

Chemicals

Five chemicals were tested in this study. They were cadmium (Cd), lead (Pb), carbon disulphide (CS_2), 1,2-dichloroethan (DCE) and vinyl chloride monomer (VCM). Embryos were exposed to the chemicals by three different routes: (1) Direct exposure – embryos explanted from untreated rats were cultured with chemicals added directly to the medium, to study the developmental toxicity of direct exposure. (2) Indirect exposure – embryos explanted from untreated rats were cultured in serum taken from treated rats, to study the developmental toxicity of chemicals in sera from exposed donors. (3) Pre-exposure – embryos explanted from treated rats were cultured with control (untreated) medium to study the developmental toxicity of chemicals pre-exposed. These three different exposure routes were compared.

Treatment with human serum

All human serum samples were analyzed for glucose concentration by the glucose oxidase method, and were adjusted to a final concentration of 3 mg/ml. In addition, human serum was prepared as a culture medium by the procedures used for preparing rat serum, including immediate centrifugation after withdrawal, heat inactivation for 30 min at 56°C, sterile filtration through an Millipore filter, and supplementation with streptomycin sulfate (0.66 mg/ml) and penicillin G potassium (0.0006 mg/ml). Human serum samples were obtained from 12 male subjects, and 18 female subjects, aged 20 to 36. Control subjects were in good health, not taking medication and not exposed to chemicals known to affect reproductive functions. Nurse serum samples were obtained from eleven female health nurses, working in oncology departments, and aged 21-42 years. The nurses had been handling ADs for 2-17 years.

Assessment of embryos

At the end of the culture period, embryos were examined under a dissecting microscope for abnormalities. Yolk sac diameters, crown-rump lengths, head lengths, and protein contents (by the Lowry method, 1951) and DNA contents (Labarca and Paigen, 1980) of embryos were measured. Cadmium analyses on sera were carried out by atomic absorption spectrophotometry. Assessment of the development of the embryos was also made using the morphological scoring systems of Brown and Fabro (1981) for rat serum as medium, and that of Klug *et al.* (1985) for human serum as medium.

Statistical analysis

Quantitative data are given as the mean plus/minus standard for each experimental group. The statistical significance of observed differences was evaluated by using Student's *t* test for quantitative, and by χ^2 for enumeration data.

Acknowledgments

This project was financially supported by the Chinese government with The Seventh Five-year Plan, the State Key-Projects for Medicine.

References

BAO, Y.S., ZHANG, X.Q and AN, B. (1979). The effects of vinyl chloride on reproductive functions of female animals and developments of embryos. *Labour Health Environ. Med. 2:* 15-19.

BARILJAK, I.R., VASILEVA, I.A. and KALINOVSKAYA, L.P. (1979). Effect of small concentration of carbon disulfide and hydrogen sulfide on intrauterine development in rats. *Arkh. Anat. Gistol. Embriol. 58:* 77-81. (In Russian). Cited from *Environmental Health Criteria 10 Carbon Disulfide.* World Health Organization, Geneva, p. 62.

BEAUDOIN, A.R. and FISFER, D.L. (1981). An *in vivo/in vitro* evaluation of teratogenic action. *Teratology 23:* 57-61.

BROWN, N.A. and FABRO, S. (1981). Quantitation of rat embryonic development *in vitro:* a morphological scoring system. *Teratology 24:* 65-78.

GUPTA, M. and BECK, F. (1983). Growth of 9.5-day rat embryos in human serum. *J. Embryol. Exp. Morphol. 76:* 1-8.

HEMMINKI, A., KYYRÖNEN, P. and LINDBOHM, M.L. (1985). Spontaneous abortions and malformations in the offspring of nurses exposed to anaesthetic gases, cytostatic drugs, and other potential hazards in hospitals, based on registered information of outcome. *J. Epidemiol. Comm. Health 39:*141-147.

JOHN, J.A., SMITH, F.A., LEONG, B.K.J. and SCHWETZ, B.A. (1977). The effects of maternally inhaled vinyl chloride on embryonal and fetal development on mice, rats, and rabbits. *Toxicol. Appl. Pharmacol. 39:* 497-513.

KIMMEL, C.A., GRANT, L.D., SLOAN, C.S. and GLADEN B.C. (1980). Chronic low-level lead toxicity in the rat. *Toxicol. Appl. Pharmacol. 56:* 28-41.

KLEIN, N.W., VOGLER, M.A., CHATOT, C.L. and PIERRO, L.J. (1980). The use of rat embryos to evaluate the teratogenic activity of serum: cadmium and cyclophosphamide. *Teratology 21:* 199-208.

KLUG, S., LEWANDOWSKY, C. and NEUBERT, D. (1985). Modification and standardization of the culture of early postimplantation embryos for toxicological studies. *Arch. Toxicol. 58:* 84-88.

LABARCA, C. and PAIGEN, K. (1980). A simple rapid and sensitive DNA assay procedure. *Anal. Biochem. 102:* 344-352.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. and RANDALL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem. 193:* 265-275.

McCLAIN, R.M. and BECKER, B.A. (1975). Teratogenicity, fetal toxicity and placental transfer of lead nitrate in rats. *Toxicol. Appl. Pharmacol. 31:* 72-82.

MCDIARMID, M. and EGAN, T. (1988). Acute occupational exposure to antineoplastic agents. *J. Occup. Med. 30:* 984-987.

NEW, D.A.T. (1978). Whole embryo culture and the study of mammalian embryos during organogenesis. *Biol. Rev. 53:* 81-123.

OESTREICHER, U., STEPHAN G. and GLATZEL, M. (1990). Chromosome and SCE analysis in peripheral lymphocytes of persons occupationally exposed to cytostatic drugs handled with and without use of safety covers. *Mutat. Res. 242:* 271-277.

RECORD, I.R., DREOSTI, I.E., MANUEL, S.J. and BUCKLEY, R.A. (1982). Interaction of cadmium and zinc in cultured rat embryos. *Life Sci. 31:* 2735-2743.

SARDAS, S., GÖK, S. and KARAKAYA, A.E. (1991). Sister chromatid exchanges in lymphocytes of nurses handling antineoplastic drugs. *Toxicol. Lett. 55:* 311-315.

SELEVAN, G.S., LINDBOHM, M.L., HORNUNG, R.W. and HEMMINKI, K. (1985). A study of occupational exposure to antineoplastic drugs and fetal loss in nurses. *N. Engl. J. Med. 313:* 1173-1178.

SKOV, T., MAARUP, B., OLSEN, J., RØRTH, M., WINTHEREIK, H. and LYNGE, E. (1992). Leukaemia and reproductive outcome among nurses handling antineoplastic drugs. *Br. J. Ind. Med. 49:* 855-861.

SORSA, M., HEMMINKI, K. and VAINIO, H. (1985). Occupational exposure to anticancer drugs-potential and real hazards. *Mutat. Res. 154:* 135-149.

STEELE, C.E., TRASLER, D.G. and NEW, D.A.T. (1983). An *in vivo/in vitro* evaluation of the teratogenic action of excess vitamin A. *Teratology 28:* 209-214.

STÜCKER, I., CAILLARD, J.F., COLLIN, R., GOUT, M., POYEN, D. and HEMON, D. (1990). Risk of spontaneous abortion among nurses handling antineoplastic drugs. *Scand. J. Work. Environ. Health 16:* 102.

TABACOVA, S. (1976). Further observation on the effect of carbon disulphide inhalation on rat embryo development. *Teratology 14:* 374-375.

TABACOVA, S., HINKOVA, L. and BALABAEVA, L. (1978). Carbon disulphide teratogenicity and postnatal effects in rat. *Toxicol. Lett. 2:* 129-133.

UNGVARY, G.Y. (1978). Effects of vinyl chloride exposure alone and in combination with trypan blue applied systematically during all thirds of pregnancy on the fetuses of CFY rats. *Toxicology 11:* 45.

VOZOVAYA, M.A. (1976).The effects of benzine and dichloroethane in low level alone or combinatively on reproductive functions of animals. *J. Gig. Sanit. 6:* 100. (In Russian).

VOZOVAYA, M.A. and MALYAROVA, L.K. (1975). The study on mechanisms of the effect of 1,2-dichloroethane on fetuses of experimental animals. *J. Gig. Sanit. 6:* 94-95. (In Russian).

WANG, Y.L. and BAO, Y.S. (1988). The studies on the effects of carbon disulphide on pregnant rats and the levels of zinc and copper in serum of female workers. *J. Health Toxicol. 2:* 146-149.

WARNER, C.W., SADLER, T.W., TULIS, S.A. and SMITH, M.K. (1984). Zinc amelioration of cadmium-induced teratogenesis *in vitro. Teratology 30:* 47-53.

ZHANG, F., ZHAO, S.F. and BAO, Y.S. (1992). Application of whole embryo culture system to detect the embryo toxicity of lead. *Chinese J. Ind. Hyg. Occup. Dis. 10:* 273-276.

ZHANG, X.C., ZHAO, S.F. and BAO, Y.S. (1992). Application of whole embryo culture morphological score method to evaluate the developmental toxicity of vinyl chloride. *J. Health Toxicol. 1:* 56-57.

ZHAO, S.F., ZHANG, X.C. and BAO, Y.S. (1984). The study on the effects of 1,2-dichloroethane on the development of mice. *Chinese J. Ind. Hyg. Occup. Dis. 2:* 343-346.

ZHAO, S.F., ZHANG, X.C. and BAO, Y.S. (1989). The study on the effects of 1,2-dichloroethane on reproductive function. *Chinese J. Prevent. Med. 23:* 199-202.

ZHAO, S.F., ZHANG, X.C., JIANG, J.G., HUANG, Y., MA, S.L., CHEN, M.H., CHEN, R.X., LI, G.Y. and BAO, Y.S. (1993). The effects of occupational exposure to antineoplastic drugs on embryos and fetuses in nurses during pregnancy. *Chinese J. Ind. Hyg. Occup. Dis. 11:* 139-142.

ZHAO, S.F., ZHANG, X.C., ZHANG, L.F. and BAO, Y.S. (1992). Study of the developmental toxicity of carbon disulphide *in vitro. Ind. Health Occup. Dis. 18:* 75-78.

ZHOU, S.S., JIANG, H., SHI, Z.M., XU, Q., YAN, C.H. and HE, J.G. (1990). The study on effects of cadmium on the development and function of rats fetuses *J. Health Toxicol. 4:* 14.