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Aroclor-1254® Effects on the In Vitro Development of 8-Cell Mouse Embryos

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Polychlorinated biphenyls (PCBs) were first synthesized in 1881 and commercial production began in the United States in 1929. Since their first commercial production, an estimated 1.2 million tons of PCBs have been produced worldwide (Tanabe 1988). Their extensive use, together with their physical/chemical properties, led to their extensive distribution in the environment in the late 1960s. Due to the widespread occurrence of PCBs in the ecosystem and their potential adverse effects on animals, including humans, significant efforts have been invested by government and industry to understand how PCBs function and what hazards could occur from their presence in the environment.

Maternal consumption of PCB contaminated fish in humans has been reported to cause reduced birth weight, diminished head circumference, and reduced gestational age (Fein et al. 1984; Swain 1991). Exposure to PCBs has caused adverse effects on reproduction in a wide range of laboratory animals. In rodents, these effects have included embryo and fetal toxicity, decreased fertility in both males and females, and decreased survival of offspring (EPA 1980; Sager 1983; Sager et al. 1987; Golub et al. 1991). Previous studies in our laboratory (Kholkute et al. 1994a) demonstrated that Aroclor-1221, 1254, and 1268 adversely affected in vitro fertilization in mice; Aroclor-1254 being the most toxic. The primary objective of this study was to investigate the effects of Aroclor-1254 on the development of the mouse embryo beyond the 8-cell stage.

MATERIALS AND METHODS

Female C57BL/6J and male DBA/2J mice both eight weeks old were used for the production of 8-cell embryos. They were purchased from The Jackson Laboratory (Bar Harbor, ME).

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Mice were housed in Plexiglass boxes and provided Teklad Rodent diet® (Madison, WI) and water ad libitum. All animals were kept at a 12 hour light:dark photoperiod and maintained in an air conditioned room at 23 ± 2° C. The housing, maintenance and care conditions were in accord with state and federal regulations.

Aroclor-1254 was purchased from AccuStandard, Inc. (New Haven, CT), dissolved in ethyl alcohol, and serially diluted in culture medium to obtain the desired concentrations. Three different concentrations of A-1254 (0.1, 1.0, and 10 µg/mL) were studied; control culture dishes contained Eagle's Minimal Essential Medium (MEM, Sigma Chemical Co., St. Louis, MO).

For embryo culture, MEM was supplemented with 0.075 g/L penicillin-G sodium salt, 0.075 g/L, streptomycin sulfate, 2.2 g/L sodium bicarbonate, and 0.1091 g/L calcium lactate. Brinster's medium for oocyte culture with 0.4% bovine serum albumin (BMOC-3, Gibco, Grand Island, NY) was used for embryo collection. The same medium was used in the outer well of Falcon 3037 organ tissue culture dishes (Lincoln Park, NY). The MEM medium was prepared with cell culture-grade distilled water (Gibco, Grand Island, NY) sterilized using a 0.22 µm filter (Millipore, Bedford, NY) and aliquots were stored at 4°C. The pH of the medium was 7.30 to 7.45. Fresh media was prepared every third week.

Females were superovulated by an intraperitoneal (ip) injection of 10 IU of pregnant mare serum gonadotropin followed by 10 IU of human chorionic gonadotropin (hCG, both from Sigma Chemical Co., St. Louis, MO) 46-48 hours later. Immediately following hCG injection, females were housed with males overnight at a ratio of one to one. Females were separated from males the next morning, checked for vaginal plug, and placed in individual boxes with food and water.

Culture dishes were prepared using BMOC-3 (3ml) and MEM (1 ml) in the outer and inner wells, respectively, and various concentrations of A-1254 and the control. The culture dishes were then equilibrated overnight in an incubator with 5% CO_2 in air at 37°C.

Eight-cell embryos were collected from mated females at approximately 57 hours after hCG injections. Female mice were sacrificed by cervical dislocation and both oviducts and uterine horns removed and placed in a Costar cell culture cluster dish (Costar Corporation, Cambridge MA) containing 2 ml of BMOC-3 medium. Embryos were removed by tearing the oviduct at several points along its length with fine forceps and flushing the isthmus of the oviducts

through the uterine horn using BMOC-3 media in a 1 ml syringe attached to a 25-gauge (5/8 inch) hypodermic needle. Embryos were collected using a 10 µl disposable micro-pipette with a mouthpiece adaptor (Dade Diagnostic, Inc., Aguada PR). The embryos were then washed once in fresh MEM.

For every replication, three concentrations of Aroclor-1254 (0.1, 1.0, and 10.0 µg/mL) were prepared. There was a minimum of six replications of each treatment. Only embryos at the 8-cell stage, not compacted, and showing normal morphology, with no signs of degeneration or abnormalities, were used. From 6-10 embryos (in a single dish) were used with each concentration. The culture dishes were placed in the incubator (5% CO_2 in air at 37°C) and observations were made every 24 hours up to 96 hours to determine the number of embryos that developed further, as indicated by the presence of compaction, morula, or blastocyst stages.

Individual Chi square test (x^2) was performed on the data in order to determine significant differences ($P < 0.05$) between the treatment and control groups.

RESULTS AND DISCUSSION

The results in these studies demonstrated a significant effect of Aroclor-1254 on the development of the 8-cell mouse embryo, specifically at 48 hours for all the concentrations (0.1, 1.0, and 10 µg/mL) of 1254. Only the concentration of 1.0 µg/mL of 1254 at 72 hours indicated a significant effect on development to the blastocyst stage. Some embryos observed were degenerated, abnormal, or dying. These embryos showed a variety of morphological abnormalities such as fragmentation,. irregular blastomeres, and cracked, empty zonae pellucidae. As expected, the number of degenerated embryos gradually increased as the period of incubation increased from 24 to 96 hours.

There was no significant effect on the 8-cell embryo during the first 24 hours of culture (Table 1). At this stage the embryo is just starting the process of compaction. The blastomeres are distinct, but gap-junction communications are established between the blastomeres as compaction occurs.

These gap-junctions serve in the transformation of developmental information among the blastomeres (Ziomek and Johnson 1981). The lack of this communication between the blastomeres during the first 24 hours of culture could be the reason that Aroclor-1254 did not

Table 1. Embryo development to the compacted 8-cell stage during the first 24 hours of in vitro culture followng exposure to A-1254[1].

Group	Total embryos	Total compacted	Percent development
Control	69	44	64
0.1 µg/mL 1254	66	51	77
1.0 µg/mL 1254	62	42	68
10.0 µg/mL 1254	56	27	48
Totals	253	164	--

[1]Chi-square analysis revealed no significant difference between any of the treatments and the control (P>0.05).

cause a significant effect on development. The disadvantage of not having gap-junctional communication at this particular stage may turn into an advantage for the embryo, granting some degree of protection (at 24 hours) against the toxic effect of Aroclor-1254. The 8-cell embryos at 48 hours are fully compacted. During compaction, cells flatten upon one another to maximize intercellular contact, establishing gap-junctional communications for the first time. During the stage of compaction, polarization of the blastomeres first becomes evident

Table 2. Embryo development to the morula stage at 48 hours of in vitro culture following exposure to A-1254[1].

Group	Total embryos	Total morulae	Percent development
Control	44	40	91
0.1 µg/mL 1254	51	26	51[1]
1.0 µg/mL 1254	42	18	43[1]
10.0 µg/mL 1254	27	15	56[1]
Totals	164	99	--

'Chi-square analysis revealed significant difference from the control (P<0.001).

(Ziomek and Johnson 1981). At 48 hours, the results indicated a significant effect of the A-1254 on the morula stage at all concentrations (Table 2). At 72 hours there was no significant effect

Table 3. Embryo development to the blastocyst stage at 72 hours of in vitro culture following exposure to A-1254.

Group	Total embryo	Total blastocyts	Percent development
Control	40	23	58
0.1 µg/mL 1254	26	19	73
1.0 µg/mL 1254[1]	18	18	100[1]
10.0 µg/mL 1254	15	10	67
Totals	99	70	--

[1]Chi-square analysis revealed significant difference from the control ($P < 0.001$).

except for the 1.0 µg/ml level of A-1254 (Table 3). This suggests that the morula at 48 hours is more susceptible to the toxic effect of Aroclor-1254 . In the case of the blastocyst at 72 hours, only the concentration of 1.0 µg/ml of Aroclor-1254 revealed a significant difference ($P<0.001$). Considering that 100% development occurred only at this concentration and that an effect is usually expected at higher concentration, suggests that this significant effect is coincidental. Since there was a significant effect at 48 hours, most of the damage to the embryo that could affect its development, had already occurred. At 96 hours, there was no significant effect ($P>0.05$) on the percent of embryos hatched (Table 4).

Table 4. Embryo development to the hatching stage at 96 hours of in vitro culture following exposure to A-1254.[1]

Group	Total embryos	Total hatched	Percent development
Control	23	5	22
0.1 µg/mL 1254	19	4	21
1.0 µg/mL 1254	18	1	6
10.0 µg/mL 1254	10	0	0
Totals	70	10	--

[1]Chi-square analysis revealed no significant differences from the control ($P>0.05$).

During this particular stage of development, the embryo is ready to start the process of implantation. Another mechanism by which Aroclor-1254 could affect the development of the embryos is by reducing the number of gap junctions. Studies conducted by Krutovskikh et al. (1995) using four different tumor-promoting agents, including Aroclor-1260, caused inhibition of intercellular

communication. These researchers developed a dye-transfer technique to evaluate cell-coupling function in fresh liver slices, and used it to demonstrate that inhibition of intercellular communication is associated with rat liver tumor progression. Hemming et al. (1991) used the dye-transfer technique with rat liver white blood cells to measure the ability of polychlorinated biphenyl congeners to inhibit intercellular communication. Bager et al. (1994) demonstrated that PCB 1260 dramatically reduced gap junction protein expression in rat liver after 20 weeks of promotion treatment. These studies were conducted using liver cells rather than embryos, however the function of the gap junction in both type of cells is believed to be involved in regulation of normal cell growth and differentiation. Further investigations are needed to determine the specific mechanism by which Aroclor-1254 affects the development of the embryo. The results of this study support some recent findings related to the toxicity of PCBs on the pre-implantation embryos. Kholkute et al. (1994b) examined the effect of A-1254 on the development of 2-cell embryos to the 4-cell stage at 48 hours. They found that increasing the concentration of A-1254 in the culture medium significantly reduced the progression of 2-cell embryos to the 4-cell stage or greater at 48 hours. The development of 4-cell embryos to expanded blastocyst was also significantly suppressed in those exposed to 0.1, 1.0, and 10.0 µg/mL of Aroclor-1254. Lindenau and Fischer, (1996) reported that Aroclor-1260 is embryotoxic in a dose-dependent manner . Their study was conducted on one-day-old cleavage stages and three day-old rabbit morulae. Their investigation further demonstrated the toxic effect of A-1254 at later stages than 2-4 cell embryos. At these stages (2-4 cells) the mouse embryo is more susceptible to disruption of development and changes in the culture environment.

Previous studies and ours with Aroclor-1254 indicate adverse effects on early cleavage in the mouse embryo. The 8-cell stage of the mouse embryo represents a very critical and important stage in the further development of the embryo. During the first 48 hours, at the morula stage, the embryo undergoes major physical reorganization, including compaction, the formation of gap-junctions and the process of polarization by which the components of the cell are re-organized.

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