**Effect of trichloroethylene (TCE) on female Albino mice and their early embryos**

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***Abstract***

Trichloroethylene (TCE) is readily absorbed into the body through the lungs and gastrointestinal mucosa. Exposure to TCE can occur from contamination of air, water, and food; and this contamination may be sufficient to produce adverse effects in the exposed populations. Oral administration of trichloroethylene (TCE; 0, 24 and 240 mg/kg/day) to female mice once daily for a period of 21 days before mating and till 5th and 7th day of pregnancy, caused a significant decrease in the body weight for non-pregnant females and weight gain for pregnant females. The percentage of survival and abortion were also significantly decreased by TCE treatment for non- pregnant and pregnant females. Histological examination for the obtained 6 and 8 day mice embryos from treated mothers showed some histopathological alterations in compared with the normal control embryos. These alterations indicated that TCE treatment resulted in retarded, delayed and deformed embryos. The results suggest that TCE has teratogenic and embryotoxic effects on the development of mice embryos following a short-term exposure of TCE.

***Key words: trichloroethylene; 6th and 8th day mouse embryo; early developmental stages***

1. **Introduction**

Trichloroethylene (TCE) is a chlorinated hydrocarbon, not naturally found in the environment (man-made); it is an environmental toxicant due to production, use and disposal. It is a colorless non-flammable liquid with a sweet odour. TCE is detectable in underground water sources, surface water, and the air (Ugden et al., 1983). TCE is produced in the petrochemical industry and has many uses, such as in dry cleaning operations, paint and printing ink removal, fumigation of rodents, manufacturing fluorocarbons, beverages (decaffeination of coffee), pet foods, medicine, pharmaceuticals, cosmetics and as an anaesthetic agent (Kaneko et al., 1997; IARC, 1995 and Maull et al., 1997). In addition, it is used in the pharmaceutical industry as a solvent for waxes, fats, resins, and oils and in the aerospace industry for flushing liquid oxygen. Its historical use in foods,

Trichloroethylene is readily absorbed following both oral and inhalation exposures. It is also absorbed from the gastrointestinal tract into the systemic circulation in animals. Using radiolabelled TCE, Mass balance studies indicated that mice and rats metabolized TCE at 38.10% and 15.10%, respectively, following oral administration in corn oil vehicle. For both species, the lower values were obtained following treatment with large doses in excess of 1000 mg/kg bw, implying that the rate of absorption was higher at low doses than at high doses in both species (Mitoma et al., 1985; Prout et al., 1985 and Rouisse & Chakrabarti, 1986).

Once absorbed, TCE diffuses readily across biological membranes and is widely distributed to tissues and organs via the circulatory system. Studies in animals **(Fernandez et al., 1977; Fisher et al., 1991)** and humans **(De Baere et al., 1997)** have found TCE or its metabolites in most major organs and tissues. Also, TCE may accumulate in adipose tissue because of its lipid solubility. Consequently, slow release of TCE from adipose stores might act as an internal source of exposure, ultimately resulting in longer mean residence times and bioavailability of TCE **(Dallas et al., 1991; Fisher et al., 1991)**. The oxidative metabolism of TCE occurs primarily in the liver, although it may also occur in other tissues, particularly the kidney and the lung. There are two main pathways responsible for TCE metabolism: oxidation by cytochrome P-450 and conjugation with glutathione (GSH) by glutathione-S-transferases (GSTs) **(OEHHA, 1999; Lash et al., 2000)**. In the liver, TCE is metabolized to an electrophilic epoxide intermediate, which spontaneously rearranges to chloral. Chloral is further metabolized to trichloroethanol (TCOH), trichloroethanol glucuronide (TCOG) and trichloroacetic acid (TCA). Other metabolites were mentioned including carbon dioxide, N-(hydroxyacetyl) aminoethanol and oxalic acid **(Goeptar et al., 1995)**.

 2. **Materials and methods**

* 1. ***Animals***

 Ninety virgin female and thirty fertile male albino mice weighing approximately 23-25 gm were used for experimentation. The animals were arranged into three groups, each was composed of 30 females as follows: The first group (A), in which the animals were treated under the same condition with the dose solvent (corn oil). The second group (B) and the third group (C) in which females were treated orally for three weeks (daily) with 24 mg/kg and 240 mg/kg body weight of TCE, respectively. The treated females allowed mating with normal male, and then the pregnant females received TCE for 5th and 7th day of gestation.

* 1. ***Mating procedures***

 The females which were found at the pro-oestrus stage were selected, each three of them were kept with one adult male in one cage overnight. Occurrence of copulation was established in the next morning at 9.00 a.m. by the presence of the vaginal plug and/or the presence of the sperms in the vaginal smear, and this day was considered to be the first day of pregnancy, assuming coitus took place at 1.0 a.m. on the night of mating **(Terada and Nishimura, 1975)**. The treated pregnant females receive TCE until 5th and 7th day of gestation and sacrificed for the collection of the embryos (6th and 8th day embryo).

* 1. ***trichloroethylene***

 Highest purity trichloroethylene (TCE) obtained from Sigma-Aldrich company at Cairo was used in the present work. The used dose was based upon the 50% lethal dose (LD50) value of 2402 mg/kg body weight in mice taken from the Registry for Toxic Effects of Chemical Substances (**NIOSH, 1988 and Tucker et al.1982**). The two doses of TCE investigated in the present study were 24 and 240 mg/kg (1/100 as low dose and 1/10 of LD50 as a high dose, respectively) dissolved in corn oil. Control doses consist of corn oil only. The used doses were made up immediately preceding dosing and a volume of 0.2 ml was given per animal.

* 1. ***Administration***

Mice were administered TCE using an 18-gauge, 3.8 cm curved gavage needle attached to a graded glass syringe. Food was withheld from all animals for 4 hours prior to daily administration.

* 1. ***Embryos collection***

The treated pregnant females sacrificed at 6th and 8th day of gestation for extracting the whole uterus with 6 and 8 day mouse embryo, respectively. The uterus fixed in 10% neutral buffered formalin for 24 h, washed, dehydrated, cleared, embedded, sectioned in 5 µm, stained with Ehrlichs heamatoxylin and eosin and mounted on a clean glass slides then examined.

 **Results**

**Maternal body weight change:**

The body weight was recorded at the end of the 1st, 2nd and 3rd week for treated females bofore mating (non-pregnant females) as well as females of the control group (Table 1 and Fig. 1). The percent of weight change as represented in Table 1 was statistically insignificant.

After mating, the body weight of females of each group was recorded every 6 days of gestation until day 18 of pregnancy. Most of the treated pregnant females showed little increase in body weight throughout the three periods. In contrast, the decrease in body weight of the treated pregnant females in comparison with the control was significant and is proportional with the duration and level of the used dose of TCE (Table 2 and fig. 1). In addition, the body weight gain of TCE treated pregnant females of group two (G2) at the end of day 18 of gestation was significantly decreased compared with body weight gain of group one (G1).

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| --- |
| **Weight of females before mating** |
| **Groups** | **1st Day** | **End of 1st week** | **End of 2nd weeks** | **End of 3rd weeks** |
| **No. of females** | **Mean ± SD** | **% of change** | **Mean ± SD** | **% of change** | **Mean ± SD** | **% of change** | **Mean ± SD** | **% of change** |
| **C** | 30 | 32.0 ± 0.40 | 0 | 32.6 ± 0.32 | 1.88 | 33 ± 0.32 | 3.13 | 33.9 ± 0.32 | 5.94 |
| **G1** | 30 | 32.0 ± 0.28 | 0 | 31.1 ± 0.26 | 2.81 | 30.2 ± 0.26 | 5.63 | 28.8 ± 0.26 | 10.00 |
| **G2** | 30 | 32.0 ± 0.21 | 0 | 30.2 ± 0.19 | 5.63 | 28.9 ± 0.19 | 9.67 | 26.7 ± 0.19 | 16.56 |

**Table 1: Relative percentage of body weight change for non-pregnant control and treated females with TCE.**

 **Table 2: Relative percentage of body weight change for pregnant control and treated females with TCE.**

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| --- |
| **Weight of pregnant females (after mating)** |
| **Groups** | **1st Day** | **End of 6th day** | **End of 12th day** | **End of 18th day** |
| **No. of pregnant females** | **Mean ± SD** | **% of change** | **Mean ± SD** | **% of change** | **Mean ± SD** | **% of change** | **Mean ± SD** | **% of change** |
| **C** | 29 | 33.6 ± 0.42 | 0 | 35.9 ± 1.22 | 6.9 | 39.6 ± 1.22 | 17.21 | 42.1 ± 1.22 | 25.3 |
| **G1** | 20 | 28.4 ± 0.75 | 0 | 30.9 ± 2.04 | 8.8 | 32.3 ± 2.04 | 13.3 | 33.2 ± 2.04 | 16.55 |
| **G2** | 12 | 26.1 ± 0.85 | 0 | 27.2 ± 2.01 | 4.2 | 28.4 ± 2.01 | 8.6 | 29.5 ± 2.01 | 12.5 |

**Fig. 1: Body weight change for non-pregnant and pregnant control and treated females with TCE.**

**1.2. Percentage of survival of non-pregnant and pregnant treated females:**

Table 3 and figure 2 illustrate that the percent of survival for control, G1 and G2 group was 100%, 90% and 76.7%, respectively at the end of the treatment period before mating. One female died from group G1 on the seconed week and two females died on the third week. While, five females died from G2, one on the first week and four females on the second and third weeks (two females for each week). At the end of the treatment period the percent of survival of females treated with the high dose of TCE (240 mg/kg b.w.) were significantly decreased in contrast with the control group. In addition, the percent of survival of pregnant females of the control group on day 18 of gestation was 100%. However,the percent of survival of the treated groups (G1 and G2) was decreased to 95.2% and 85.7%, respectively (Table 3 and Fig. 2).

**Table 3: The percentage of survival and mating of control and treated females with TCE.**

|  |  |
| --- | --- |
| **% of survival** | **% of mating** |
| **Groups** | **Before mating (non-pregnant)** | **After mating (pregnant)** |
| **No. of females** | **1st  week** | **2nd week** | **3rd week** |
| **No. of dead females** | **Percentage (%)** | **No. of dead females** | **Percentage (%)** | **No. of dead females** | **Percentage (%)** | **No. of dead females** | **Percentage (%)** | **No. of survival females** | **No. of pregnant females** | **% of mating** |
| **C** | 30 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 30 | 29 | 96.7 |
| **G1** | 30 | 0 | 100 | 1 | 96.7 | 2 | 90.0 | 1 | 95.2 | 27 | 21 | 77.8 |
| **G2** | 30 | 1 | 96.7 | 2 | 90.0 | 4 | 76.7 | 2 | 85.7 | 23 | 14 | 60.9 |

**Fig. 2: The percentage of survival of non-pregnant and Fig. 3: the percentage of mating of treated females with normal males.**

**pregnant control and treated females with TCE.**

**1.3.** **Percentage of mating:**

Most of the treated females were symptomised by reduced excitability and appetite. At the end of the third week of treatment the females were allowed to mate with normal proven males. It was observed that the percent of mating for the treated females was less than the control. The percent of succeeded copulation was 77.8% for G1 and 60.9% for G2 compared with 96.7% for the control group (Table 3 and Fig. 3).

**1.4. Percentage of abortion**

The present study showed one female from the first group (G1) that treated with the low dose of TCE (24 mg/kg b.w.) and three females of the second group (G2) that treated with the high dose of TCE (240 mg/kg b.w.) were aborted during the gestation period. Howevere, the control group showed no aborted females. The abortion was confirmed by the presence of blood drops in the vagina and sudden loss in maternal body weight. The percent of abortion in the second treated group (G2) was more than that of the first group (G1) and control pregnant dams (Table 4 and Fig. 4). The mean number of feotuses was markedly reduced in treated groups.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Groups** | **No. of pregnant females** | **No. of aborted females** | **% of Aborted females** | **No. of females with alive foetuses** | **No. of foetuses / mother** |
| **Mean ± S.D** | **%** |
| **C** | 29 | 0 | 0 | 29 | 6.98 ± 0.41 | 100 |
| **G1** | 20 | 1 | 5.0 | 17 | 6.15 ± 0.23 | 88.11 |
| **G2** | 12 | 3 | 25.0 | 8 | 4.82 ± 1.55 | 69.05 |

**Table 4: the percentage of aborted treated females and fetuses/mother maternally treated with TCE.**

**Fig. 4: The percentage of aborted females, foetuses/mother, stillbirth, resorbed foetuses, and total foetal mortality.**

**6th day embryo (132 h.P.C)**

At this stage, the uterine epithelium has disappeared completely from around the circumference of the developing embryo. The epithelial basement membrane is not present and the egg-cylinder is embedded in a mass of decidual tissue and has differentiated into its basic layers (Fig. 5). The embryo consists of three regions; ectoplacental cone, extraembryonic and embryonic regions. Proximal endoderm, distal endoderm and trophectoderm are identified respectively around the central cylinder. A proamniotic cavity appears in the centre of the embryonic ectoderm, followed by another cavity in the centre of the extraembryonic ectoderm. The two cavities are joined together to form an elongated cavity later. The yolk-sac cavity is present between the proximal and distal endoderms.

The ectoplacental cone connects the embryo ventrally and extended dorsally towards the lumen of the uterus. This cone is formed by proliferation of the dorsal portion of the extraembryonic ectoderm and trophectoderm that found at this region. This structure is a mass of round or polygonal cells with ovoid nuclei. It exhibits a porous appearance at this upper extremity where mother blood fills its interspaces.

The proximal endoderm is identified around the egg-cylinder and lines the proximal wall of the yolk-sac cavity. This layer is consisted of cuboidal or low columnar cells found mainly at the extraembryonic region of the embryo. Their cytoplasm is moderately eosinophilic with basically located basophilic stained nuclei. Some vacuoles of different sizes are found in the supra-nuclear cytoplasm. The proximal endodermal cells are arranged in one layer with unbound surfaces. These cells are gradually changing their shape in the direction of the antimesometrial pole of the embryo. They become flattened or cylindrical in shape.

The distal endoderm is extended from the proximal endoderm over the inner surface of the trophectoderm and lines the distal wall of the yolk-sac cavity (Fig. 5). This layer of the endoderm is consisted from a single raw of flattened cells having nearly the same stainability as the trophectodermal cells, thus it is not easy to differentiate between the two types.

The extraembryonic ectoderm is consisted of rounded or cuboidal cells lying at the mesometrial pole of the embryo. These cells are arranged in 1-4 layers of cells around the proamniotic cavity. The cytoplasm of these cells is slightly basophilic with round basophilic nuclei. Few degenerated or dead cells with pyknotic nuclei are present in this layer and cellular remnants are seen in the proamniotic cavity.

The embryonic ectoderm consists of some layers (2-4 rows) of cuboidal or low columnar or cylindrical cells lying around the proamniotic cavity at this region of the embryo. These ectodermal cells are less basophilic with oval nuclei and one or two densely basophilic nucleoli. Also, dead or degenerated cells with dense basophilic and pyknotic nuclei are present (Fig. 5). Few deteriorated cells with disintegrated cytoplasm are found at the luminal surface of the embryonic ectoderm and within the proamniotic cavity.

The trophectoderm constitutes the outer layer of the egg-cylinder. This layer is observed in direct contact with the decidual tissue of the mother. The trophectodermal cells are flattened or cylindrical in shape with less eosinophilic cytoplasm and large oval basophilic nuclei. These cells are indistinguishable from the distal endoderm in some regions and from the decidual cells in other regions (Fig. 5).

A cylindrical embryo of group (B) shown in figure 6 is consisted of a central mass of ectodermal cells, extraembryonic and embryonic cells. These cells appeared normal with eosinophilic cytoplasm and basophilic rounded nuclei. In the middle area of the ectodermal mass a few number of cells showed less eosinophilic cytoplasm and dense basophilic, probably, pyknotic nuclei. Some proximal endodermal cells at the lateral dorsal region of the egg-cylinder were spherical or flattened instead of being cuboidal or low columnar with moderately eosinophilic stained cytoplasm and dense basophilic nuclei around which halo spaces are seen. Several decidual cells around the embryo exhibited less eosinophilic cytoplasm, large dense basophilic nuclei and halo spaces.

The embryonic cells at Fig. 7 were less eosinophilic and the nuclei were less basophilic. Several degenerated cells with pyknotic nuclei were shown in all layers of the embryo. The number of dead cells was increased than that observed in normal pregnancy. In addition, vacuolated decidual cells were demonstrated with shrinkage cytoplasm and pyknotic nuclei. Few deteriorated proximal endodermal cells were found at the dorso-lateral side of the embryo (Fig. 7).

Microscopical examination of sagittal sections in the embryos obtained from dams treated with the high dose of TCE (240 mg/kg b.w.) showed some deformed embryos with defined structures. The ectoplacental cone was undeveloped with indefinite cellular appearance. A large empty space was seen in its centre. Dead cells with pyknotic nuclei were observed in the extraembryonic ectoderm. Also, vacuolated cells with shrinkage cytoplasm were evidented. The proximal endoderm looks normal at the mesometrial pole of the embryo and disrupted at the antimesometrial pole. The most striking feature in this specimen was the deformation of the embryonic ectoderm that will form the embryo proper. It comprised numerous vacuolated cells with small dense nuclei, dead and disintegrated embryonic cells at its luminal surface and somewhat wider proamniotic cavity. Small haemorrhagic patches were seen very near to the undeveloped ectoplacental cone and at the antimesometrial pole of the egg-cylinder (Fig. 8).

At the high dose level of TCE, completely degenerated or destructed embryos were investigated (Fig. 9). Cells of the ectoplacental cone were basophilicaly stained and the normal porous appearance of these cells was disappeared. Vacuoles of different sizes and small haemorrhagic patches were also observed. Most of the endodermal cells were highly vacuolated with degenerated nuclei. The proximal endodermal cells were disorganized and they were round in appearance and found around the circumference of the degenerated egg-cylinder. Some of these cells were degenerated with chromatolytic nuclei. The distal endodermal cells were ill-defined; some with pyknotic nuclei. The decidua around the embryo showed many degenerated round cells with large vacuoles and pyknotic nuclei. Phagocytic cells were increased in the deciduoma very near to the degenerated embryo. Large vacuoles or empty spaces were seen around the implanting embryo separating it from the surrounding deciduoma probably preventing the arrival of nourishment from the mother tissues at this stage of pregnancy (Fig. 9).

**8th day embryo (180h p.c.):**

The mouse embryo at this stage in the form of egg-cylinder is much more developed and increased in size than the previous described embryo at day 6 of pregnancy. The embryo reaches about 300 µm in diameter. The embryo is seen pendanted in the uterine tissue by the ectoplacental cone (Fig. 10). The ectoplacental cone is formed from the dorsalward proliferation of cells of the extraembryonic ectoderm. Cells of the cone are basophilic stained with round or oval nuclei and prominent nucleoli. Also, the trophectoderm cells at this region take part in the formation of this structure. Some cells of the ectoplacental cone are vacuolated with dense basophilic and pyknotic nuclei representing degenerating cells in the cone. The ectoplacental cone invades the maternal tissues at this region, rupturing blood vessels in its path.

The developed cylindrical embryo at this stage filled the yolk-sac cavity leaving a narrow space between the proximal and distal endoderm, which is obliterated later on. The egg-cylinder shows many changes in its form and architecture when compared with the previous studied 6-day normal embryo at 132h p.c. (Figs 10 & 11). The examined 8-day embryo (180h p.c.) consists of three regions, extraembryonic, embryonic between which the extracoelomic region is present. In addition, it acquires three embryonic cavities; ectoplacental cone cavity, exocoelomic cavity and amniotic cavity.

The extraembryonic region is observed dorsaly just below the well-formed ectoplacental cone. It consists of a layer of extraembryonic ectoderm around a semicrescent slit or cavity represents the remain of the ectoplacental cone cavity (Fig. 10). Cells of this layer are low columnar with basophilic cytoplasm and spherical or ovoid nuclei. The chromatin material accumulates into one or two masses within the nucleus. Cells with apoptic nuclei are also investigated in this layer (Fig. 12). A layer of flattened or extended mesodermal cells is seen lining the ventral side of the extraembryonic ectoderm forming the chorion, which is one of the embryonic membranes. Also, some mesodermal cells are pushing their way between the extraembryonic ectoderm and the adjacent endoderm taking part in the formation of the yolk-sac membrane which envelops the embryo. In such case, the embryo is separated from the mother tissue by the yolk-sac and Reichert's membranes (Fig. 12).

The proximal endoderm of the extraembryonic and the exocoelomic regions is consisted of columnar cells with basally located basophilic nuclei. Vacuoles of different sizes are found in the supranuclear cytoplasm. A heavy eosinophilic material is found at the luminal surface of these cells (Fig. 13). This material is maternaly in origin.

The embryonic ectodermal region of the egg-cylinder is found at its antimesometrial pole. This layer of the ectoderm forms the embryo proper. The ectoderm at this region consists of stratified columnar cells exhibited basophilic cytoplasm with oval nuclei and one or two densely stained nucleoli. Degenerated cells are investigated at the luminal surface of the embryonic ectoderm. These cells showed dense basophilic nuclei and deteriorated cytoplasm (Fig. 14). The embryonic ectoderm at the posterior end of the embryo is thickened as a strip of cells forming the primitive streak, establishing the anterior-posterior axis of the embryo. Dorsal to the primitive streak is the posterior amniotic fold, which is formed previously from the proliferated mesoderm. Within this fold, a cavity is formed and the fold with its cavity expanded more and more dividing the proamniotic cavity into the three cavities mentioned before. The ectodermal cells found at the dorsal side of the embryonic ectoderm become elongated and flattened forming together with the extending mesodermal cells, the amnion which is the second embryonic membrane (Fig. 14).

The mesoderm found between the flattened proximal endodermal cells and the embryonic ectodermal cells extended dorso-laterally on both sides of the egg-cylinder. The mesoderm is consisted of scattered mesenchyme cells basophilic stained showing round nuclei and one or two dense nucleoli. At the posterior end of the embryo, a mass of mesodermal cells is bluging into the exocoelomic cavity forming the allantois, which is the third embryonic membrane (Fig. 14). In addition, a small group of mesodermal cells accumulates at the anterior end of the embryo forming a fold within the exocoelomic cavity, the anterior amniotic fold; therefore, the anterior of the developing embryo is defined. By this mean the anterior-posterior axis of the embryo is more pronounced.

The middle region of the egg-cylinder is the exocoelomic region consists mainly of columnar proximal endodermal cells, at the outside, facing the yolk-sac cavity and mesenchyme cells that lining the exocoelomic cavity. The exocoelomic mesodermal cells are elongated or thin cells with round nuclei located in the middle of the cytoplasm. Few deteriorated round cells with pyknotic nuclei are oftenely observed in the exocoelomic cavity; most probably, they are of mesodermal origin.

The decidua contains mono and binucleate cells however, tri and even tetranucleate cells are oftenely present. In the decidua the blood looses from ruptured blood capillaries pathing to the embryo almost directly or through the trophectoderm and the distal endoderm.

Cylindrical embryos obtained from pregnant mothers treated for 21 days before pregnancy and for 7 days during pregnancy with low dose of TCE (24 mg/kg b.w.) showed some variations in the form and structure from the normal (control) embryos (Fig. 11). Two main cavities were observed; exocoelomic and proamniotic cavities. This embryo was retarded than that obtained from normal pregnancy. It showed necrotic mass of cells in the extraembryonic region. This mass displayed highly vacuolated cells and cell remnants. Large densely stained cells probably phagocytes were also present. Most probably, this necrotic mass comprised degenerated cells from the proximal endoderm and the adjacent extraembryonic ectoderm. Few degenerated cells were seen in the yolk-sac cavity between the proximal and distal endoderm (Figs 15&16).

The embryonic ectoderm showed numerous vacuolated cells with dense nuclei, the mesoderm was consisted of dispersed unidentifiable cells found at one lateral side of the embryonic ectoderm and not around it. This picture was not found in a comparable stage of normal pregnancy. Dissociated and disrupted proximal endodermal cells were found mainly at the junction between the extraembryonic and embryonic ectoderm. The decidua around the embryo looks normal and pools of maternal blood were shown. Some decidual cells were vacuolated with densely stained nuclei (Fig. 30)

Examination of 8-day embryos (180h p.c.) obtained from pregnant mice treated previously before mating for 21 days and during pregnancy for 7 days with high dose of TCE (240 mg/kg b.w.) showed that most embryos are of normal histoarchitecture as those obtained from normal pregnancy (Fig. 24). However, retarded, deformed and sometimes degenerated embryos were demonstrated.

Figure 32 illustrates a retarded embryo, the structure of which represents a stage of development between day 6 and day 8 of pregnancy. The ectochorionic cavity was separated from the proamniotic one by a thick layer of the embryonic ectoderm. The exocoelomic cavity and the allantois were absent. The mesoderm was found at the junction between the extraembryonic and embryonic ectoderm, extending downwards between the embryonic ectoderm and the proximal endoderm. Several embryonic ectodermal cells showed vacuoles and dense pyknotic nuclei. Cells of the ectoplacental cone were highly vacuolated and the underneath extraembryonic ectoderm showed disorganized cells. Large densely stained cells were observed in the decidua around the embryo probably representing phagocytes. The blood islands, found around normal embryos at this stage of development, were absent.

Some other egg-cylinders obtained from high dose TCE treatment exhibited a deformed structure (Fig. 33). Again, the general architecture of the egg-cylinder represents a stage of development before day 8 of normal pregnancy. The histological appearance of this embryo showed unidentifiable layers and cells. The ectoplacental cone is represented by a small group of cells found at the mesometrial pole of the embryo. Abnormal tongue like-structure from the proximal endoderm consisted of degenerated cells was seen in the dilated yolk-sac cavity. The extraembryonic and embryonic ectoderm consisted of disorganized cells. The mesodermal cells appear at one side of the embryo extending downwards. The posterior amniotic fold was not fully expanded within which small exocoelomic cavity was present. The decidual cells displayed eosinophilic cytoplasm and dense nuclei, some cells showed up to four nuclei in cytoplasm. Maternal blood was not found around the deformed embryo.

Other embryos showed densely stained undistinguishable layers and cells. In such case, the embryo had lost its characteristic histological features. Most cells were dead or dying with very dense pyknotic nuclei (Fig. 34). Two cavities were present in this embryo representing the ectoplacental cone and proamniotic cavities. In the decidua, many vacuolated cells with dense nuclei were recognized and some large cells probably phagocytes. Heamorrhagic patches were shown around this embryo, probably represent an initial stage of abortion.

The results obtained from embryos of day 6 and 8 of pregnancy showed that TCE has embryotoxic and teratogenic effects on postimplantation embryos and the degree of these effects dependent upon the level and duration of the dose. Whereas, the sever effects appeared in G2 more than that of G1 in contrast with the control group.

Discussion:

**TCE** vapors have been shown to cross the human placenta during childbirth **(Laham, 1970),** with experiments in rats confirming this finding **(Withey & Karpinski, 1985)**. In particular, **Laham (1970)** reported determinations of **TCE** concentrations in maternal and fetal blood following administration of **TCE** vapors intermittently and at birth.

**Withey & Karpinski (1985)** exposed pregnant rats to **TCE** vapors (302, 1,040, 1,559, or 2,088 ppm for **5** hours) on **17th** day of gestation and concentrations of **TCE** in maternal and fetal blood were determined. At all concentrations, **TCE** concentration in fetal blood was approximately one-third of the concentration in corresponding maternal blood.

In the present study, the body weight was recorded at the end of **1st, 2nd** and **3rd** week for treated females bofore mating (non-pregnant females) and after mating, the body weight of pregnant females of each group was recorded every **6** days of gestation until day **18** of pregnancy. As well as females of the control group in two cases. Most of the treated females showed significantly low increase in the body weight. Where, the non-pregnant females of **G1** showed loss appetite and accordingly a decrease in body weight than the control. The females of **G**2 showed loss of both appetite and activity. The decrease in body weight is proportional with the duration and level of dose. Most of the treated pregnant females showed little increase in body weight gain throughout the three periods. the body weight gain of **TCE** treated pregnant females of group two **(G2)** at the end of day 18 of gestation was significantly decreased compared with body weight gain of group one **(G1)**.

Exposures to **TCE** during the prenatal period have been reported to induce neurobehavioral alterations in rat pups, including changes in long-term exploratory and locomotor behavior, altered glucose uptake and metabolism in the neonatal brain, and decreases in myelin **(Taylor *et al.,* 1985 and Isaacson *et al.,* 1990)**.

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In the present investigation, embryos at the 6th day of pregnancy "129 hrs p.c." ectoplacental cone stage maternally treated with TCE show more or less delay in growth; as distal endoderm is not yet differentiated and degeneration of the uterine epithelial cells.

At the 8th day of pregnancy "182 hrs.p.c." embryo prober stage maternally treated with TCE showed a considerable retardation in their growth. Whereas, ectoplacental cone is incomplete. As well as, the endoderm was not differentiated into distal and proximal endoderm. The degenerated decidua is represented by vacuolated cytoplasm, pyknotic nucleus and haemorhage.

In the present investigation, the percent of survival for control, **G1** and **G2** group was **100%, 90%** and **76.7%**, respectively at the end of the treatment period before mating. The percent of survival of pregnant females of the control group on day 18 of gestation was **100%.** However,the percent of survival of the treated groups **(G1** and **G2)** was decreased to **95.2%** and **85.7%**, respectively. The percent of survival of **G2** were significantly decreased in contrast with the control group.

**Manson *et al.* (1984)** reported that exposure of ratsto **TCE** by gavage in corn oil at doses of 0, 10, 100, or 1000 mg/kg/day for 2 weeks prior to and throughout mating to day 21 of gestation exhibited increased maternal mortality, decreased maternal weight gain, and decreased neonatal survival in the high-dose group.

Both **TCA** and **DCA** exposure were associated with a substantial increase in implantation and resorption sites and decreased fetal weight **(Smith *et al.,* 1989 & 1992).** These effects were observed in the absence of maternal toxicity.

In the present work, most of the treated females were symptomised by reduced excitability and appetite. At the end of the third week of treatment the females were allowed to mate with normal proven males. It was observed that the percent of mating for the treated females was less than the control. The percent of succeeded copulation was **77.8%** for **G1** and **60.9%** for **G2** compared with **96.7%** for the control group.

These result disagree with **Manson *et al.* (1984),** they suggested that TCE had no influence on mating success.

**Fredricksson *et al.* (1993)** suggested thatexposure to TCE during the prenatal period have been reported to change adult behavior with prenatal exposure to 50 mg/kg TCE.

The present study showed one female from the first group **(G1)** that treated with the low dose of **TCE (24 mg/kg b.w.)** and three females of the second group **(G2)** that treated with the high dose of **TCE (240 mg/kg b.w.)** were aborted during the gestation period. Howevere, the control group showed no aborted females. The percent of abortion in the second treated group **(G2)** was more than that of the first group **(G1)** and control pregnant dams.The mean number of feotuses was markedly reduced in treated groups.

The above mentioned results agree with those obtained by **(Windham *et al.,* 1991; Taskinen *et al.,* 1994 and ATSDR, 2001)** they reported that exposure to TCE Increased risk of spontaneous abortion among frequently-exposed women, but disagree with **(Goldberg *et al.,* 1990; Lindbohm *et al.,* 1990 and ATSDR, 2006& 2008)** they reported that exposure to TCE hasno risk of spontaneous abortion after paternal exposure.

In human studies of prenatal TCE exposure, increased risk of spontaneous abortion was observed in some studies **(Windham *et al.,* 1991; Taskinen *et al.,* 1994 and ATSDR, 2001)**, but not in others **(Lagakos *et al.,* 1986; Taskinen *et al.,* 1989; Goldberg *et al.,* 1990; Lindbohm *et al.,* 1990 and ATSDR, 2008)**. In addition, perinatal deaths were observed after 1970, but not before 1970 (Lagakos et al., 1986). In rodent studies that examined offspring viability and survival, there was an indication that TCE exposure may have resulted in increased pre-and/or postimplantation loss **(Healy *et al.,* 1982; Narotsky & Kavlock, 1995 and Kumar *et al.,* 2000),** and in reductions in live pups born as well as in postnatal and postweaning survival **(George *et al.,* 1985 & 1986)**.

In the present investigation, embryos at the 6th day of pregnancy "129 hrs p.c." ectoplacental cone stage maternally treated with TCE show more or less delay in growth; as distal endoderm is not yet differentiated and degeneration of the uterine epithelial cells.

At the 8th day of pregnancy "182 hrs.p.c." embryo prober stage maternally treated with TCE showed a considerable retardation in their growth. Whereas, ectoplacental cone is incomplete. As well as, the endoderm was not differentiated into distal and proximal endoderm. The degenerated decidua is represented by vacuolated cytoplasm, pyknotic nucleus and haemorhage.