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STUDIES ON THE TERATOGENICITY OF PVC

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Vinyl chloride (VC) is ranking 23rd among the 50 most widely used industrial chemicals, and has an annual production of approximately 8 billion kgs in the world (Reynolds et al., 1975). To give an idea of the considerable industrial, economic and social importance of polyvinyl chloride (PVC) manufactured from VC I quote from the Chem. Eng. News in 1975: "If the polyvinyl chloride products industry were to be shut down before substitutes could be found and their manufacture begun, perhaps 2 million jobs and 75 billion dollars worth of production would be lost" (Boden, 1976).

The importance of the chemical explains that once the suspicion of the oncogenic effect of VC — thought of as an inert gas sofar — was established, a series of studies were started on the carcinogenic, mutagenic and teratogenic effects of VC. Viola et al. (1971), then Maltoni (1973), Maltoni and Lefemine (1974, 1975) confirmed the oncogenic effects of VC in mice, rats and hamsters, by showing that VC exposure induced malignant neoplasms in different tissues in a dose-dependent fashion, more or less often, sooner or later after exposure. Malignant tumours could be found in any of the organs but most of them were seen in the liver, brain and lymphatic nodes. It was recognized that the VC-induced liver tumours in experimental animals have the structure of an angiosarcoma. The first human case of angiosarcoma with a probable causal relationship with VC exposure was found in 1973 (Creech and Johnson, 1974) suggesting an increased incidence in VC workers of the angiosarcoma of the liver — an otherwise rare tumour in men. The internal report of the WHO's International Agency for Research on Cancer gave account of 14 VC-induced liver angiosarcoma cases in June 1974, while 43 cases were reported on in the 1975 January issue. The increased incidence of VC-induced angiosarcoma of the liver in men has been confirmed by a number of studies (Thomas and Popper, 1975; Makk et al., 1976; Smith et al., 1976). A higher incidence of other tumours than in control populations has also been reported (Tabershaw and Gaffey, 1974; Lange et al., 1975; Infante et al., 1976c; Waxweiler et al., 1976).

The finding of the oncogenic effect of VC was shortly followed by reports on the mutagenic effects of its metabolites chloroethylene oxide and chloroacetaldehyde in microbial assay systems (Rannug et al., 1974; Bartsch et al., 1975; Malaveille et al., 1975; Loprieno et al., 1976) and on mammalian cells (Huberman et al., 1975). At the same time an increased incidence of chromosome aberrations in PVC workers was described simultaneously in several countries (Ducatman et al., 1975; Purchase et al., 1975; Funes-Cravioto et al., 1976; Hausteen et al., 1976; Szentesi et al., 1976).

These findings led to studies on the possible teratogenic and embryotoxic effects of the agent. Studies in human epidemiology were reported by Infante et al. in 1976 (a, b, c, d). They followed the incidence of birth defects from 1954 in 3 Ohio communities, with 12—24 000 inhabitants, where PVC polymerization plants are located. In one of them — Painesville — there are 2 plants; the second began operation in 1967. The incidence of malignant tumours of the CNS and lymphatic system in the 3 communities between 1958 and 1973 was significantly higher than that in the entire Ohio State. Similarly the incidence of congenital defects and malformations in the 3 communities was significantly above that in Ohio. Malformations of the CNS, lip and palate clefts, abnormal genital organs and clubfoot were leading but the number of other defects was increased too. With knowledge of the data reported by Infante et al. (1976a, b, c, d) Edmonds et al. (1975) reinvestigated the question. They compared the incidence of conatal CNS malformations in two hospitals located in cities with PVC poly-

merization plants, one in Pennsylvania, the other in Painesville, Ohio (the one studied by Infante et al.). No increase in CNS malformations was seen in the Pennsylvania hospital, but an increase was noted in the Painesville hospital. A detailed analysis of the Painesville data revealed that there was no correlation between the increase in CNS malformation rate and VC exposure. None of the parents of the affected infants had ever worked at the VC polymerization plants at Painesville and control mothers worked closer to the plant than those of the affected infants. In Hungary Czeizel et al. (1978) studied the incidence of congenital malformations among the offspring of 86 workers (62 males, 24 females) employed in VC production and polymerization in the Borsodi Chemical Works at Kazincbarcika, Hungary, with the interview-questionnaire method. They found one case of spina bifida among 3 cases of malformations.

Recently Edmonds et al. (1978) published the results of a new epidemiological study. For the period between 1970 and 1974 they found an increase in CNS malformations in Charleston, Kanawha County, West Virginia, where a PVC manufacturing plant is working. They studied the relationship of CNS defects and occupational or residential VC exposure of the parents. The incidence of malformations and the occupation of the parents were not related. There was an accumulation of cases at one location, but the increased VC contamination of the place could not be proved. (This observation did not entirely correlate with existing data on local patterns of wind direction and air pollution.) No correlation between the annual change of incidence of CNS malformations and the amount of annual VCM emissions was found.

Another potential noxious effect of VC exposure on the offspring is the increase in intrauterine mortality and stillbirth ratio. Based on an interview-questionnaire investigation including all polymerization workers Infante et al. (1976c) calculated the fetal mortality rates in the pregnancies of the wives. Prior to husbands' exposure, the lethal mortality rates for the control and study group were 6.9 and 6.1 per cent respectively, while subsequent to husbands' exposure the rates were 8.8 and 15.8, respectively. The difference is significant. A similar tendency was seen even when mothers with 2, 3 or 4, spontaneous abortions were not

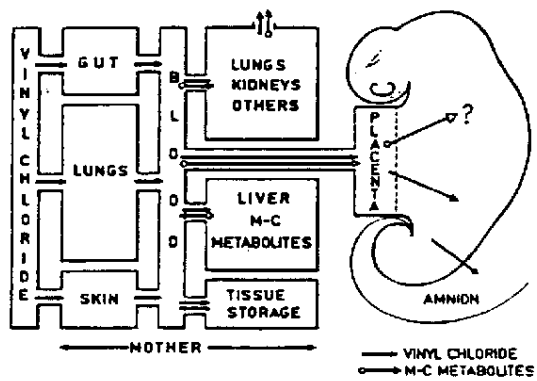

Fig. 1. Diagram of the maternal and fetal organisms exposed to VC (—). Through the lungs, gut and skin of the mother the VC is absorbed into the maternal blood stream; then VC crosses the placenta and enters the fetus as well as the amniotic fluid. In the maternal liver the VC is metabolized into mutagenic and carcinogenic metabolites (M-C, o—) — chloroethylene oxide and chloroacetaldehyde — which reach the fetus and are excreted by the maternal organism via the lungs, bile, kidneys. The VC concentration in the maternal as well as fetal blood samples depends on the atmospheric VC concentration inhaled by the pregnant mother. In spite of the fact that VC and its metabolites enter the fetus, no teratogenic effect can be observed

included. The authors suggest that the deleterious effect of VC exposition on the germ cells of the fathers is responsible for the increased fetal mortality.

Among wives of workers employed in VC production Selikoff (1974) found a fetal loss of 7—14 per 100 pregnancies, — a value higher than expected.

Czeizel et al. (1978) conducted a study in the Borsodi Chemical Works at Kazincbarcika, Hungary, where a VC as well as a PVC producing plant are operated. Fetal mortality was higher after, than before the start of employment (taken as the start of exposition). They found a twofold increase in spontaneous abortions and stillbirth rate among the wives of male workers and a 15-fold increase in the female workers. The increase in age alone does not explain the differences. The stillbirth rate and duration of exposure were correlated. In an earlier study at the same plant they found correlation between the frequency of chromosome aberrations and the duration of VC exposition (Szentesi et al., 1976).

Purchase et al. (1975) and Anderson et al. (1976) studied the VC for dominant lethal effects in male CD-1 fertile mice. No mutagenic effects of VC exposure on any maturation stages of spermatogenesis in the treated males were detected. No increase was found in post-implantation and early fetal deaths, in preimpantation egg losses and no reduction was found in fertility, i.e. VC did not produce dominlant lethal effect in mice. Short et al. (1977) reported on a similar finding with CD male rats — where also no change in postimplantation fetal losses were noted.

The mutagenic effect of VC exposure of the *Drosophila* species was studied by Verburgt and Vogel (1977). The dominant lethal test gave negative, while the recessive lethal test in short and long term experiments gave positive results. They argued that the point mutations are brought about at lower, while chromosome breaks only at higher levels of exposure to the mutagen.

In an experiment by John et al. (1977) pregnant CF-1 mice were exposed to 50 or 500 ppm of VC and pregnant SD rats and NZ white rabbits were exposed to 500 or 2500 ppm of VC for 7 hours a day during the entire period of organogenesis. Other groups exposed to the above VC concentrations for the same period were given ethanol in their drinking water. In spite of the toxic effects on the mothers produced by the exposure to higher concentrations of VC, no sign of embryonic or fetal toxic or teratogenic effects were seen in any of the three species. Alcohol treatment brought about a substantial increase in maternal and a slight fetal toxicity, but no teratogenic effect was observed after combined VC and alcohol treatment.

Our results can be summarized as follows. Pregnant CFLP mice were exposed to VC at 1000 ppm concentration for 2 hours, four times a day, for the entire period of organogenesis. An increase in fetal losses was found, but no teratogenic effect could be observed.

When pregnant CFY rats were inhaling VC for 24 hours a daye during the entire perio of organogenesis at 1000 ppm concentration, an increase in relativ reweight of the maternal liver, indicating maternal toxicity was found, but no embryotoxic orateratogenic effects were observed (Ungváry et al., 1977). A similar VC exposure of pregnant ru ts during the last third of pregnancy had no effect on the offspring; when a similar exposure was applied during the first third of pregnancy an increase in fetal mortality watunoted as well as an embry otoxic effect indicated by the weight retard ation of the feses. When a group of pregnant rats was exposed to 1000 ppm of VC during the 1—9 days of pregnancy, but was given trypan blue injections on the 7th and 8th days of gestation, the trypan blue induced increase in fetal losses and incidence of malformations were not potentiated by VC exposure (Ungváry et al., 1978). For 3 weeks before mating and during the entire pregnancy a group of rats kept on DeCarli and Lieber's alcoholic liquid diet was exposed to 1000 ppm of VC continuously for 24 hours a day, during the neurulation. No congenital malformation was seen in the offspring, but an increase in the incidence of skeletal retardation cases was found after the combined VC ethanol treatment.

One could say, that epidemiological studies in men have caused considerable concern, but that these concerns in some way are counterbalanced by the negative results of experimental studies. *One may well seek the difference between fetal losses and congenital malformations.* Most of the epidemiological studies in men including those of Edmonds et al. (1978), indicate that VC does not possess a teratogenic effect. Animal studies in three species argue also against the teratogenic effect of the agent (John et al., 1977; Ungváry et al., 1977, 1978). Though the straight extrapolation of the results from animal studies in experimental teratology to the human conditions raises considerable difficulties, careful consideration of the experimental design might substantially reduce these difficulties. One may recall, that the animal studies of Maltoni and Lefemine (1974) revealed not only the oncogenic effect of VC exposure, but indicated the induction of a particular tumor, hemangiosarcoma of the liver. Indeed, the carcinogenic property of VC in men has just been identified by the finding of the hemangiosarcoma of the liver, a very rare tumour in the human.

Moreover, considerable amounts of VC cross the placenta. The VC concentrations of the maternal as well as embryonic blood samples are correlated with the atmospheric VC concentration inhaled by the pregnant mother. VC can be found also in the amniotic fluid (Ungváry et al., 1977, 1978). Although thus far no correlation has been shown between the mutagenic and oncogenic VC metabolite concentration of maternal origin in the maternal and fetal blood (Fig. 1), it is known that VC exposure of the mother may induce transplacental tumours though these metabolites (Maltoni, 1975). Taking our finding into consideration, that VC exposure of pregnant rats for 24 hours a day, during the entire period of organogenesis did not result in an increased incidence of congenital malformations, *one may conclude, that the VC and its metabolites do not possess a teratogenic effect*, or *if they do, they do not reach the effective teratogenic concentration even in the case of continuous exposure of the mothers at high atmospheric concentration.*

It is different, however, with the fetal losses. The data of Selikoff (1974), Infante et al. (1976a, b, c, d) and Czeizel et al. (1978) leave no doubt about the increase in fetal losses subsequent to VC exposure. Still no positive response was seen in dominant lethal tests in mice, rats and the *Drosophila* species. The finding of Czeizel et al. (1978) of an increased fetal mortality in the pregnancies of female VC polymerization workers is in good agreement with our finding of increased fetal losses seen after the continuous exposure of rats to VC during the first 3rd of pregnancy, or after a frequent exposure of pregnant mice to the agent during organogenesis (Ungváry et al., 1978).

In summary, one may conclude, that the substance VC of high industrial and socioeconomic importance is most probably not a teratogenic agent. Taking, however, its carcinogenic and mutagenic effects into consideration the lack of teratogenicity does not reduce the concern raised by VC for the industrial hygiene. *The increase in embryonic and fetal mortality after exposure of the parents is a further hazard.* Since the induction of carcinogenic as well as mutagenic effects are most probably highly dependent on the duration and the level of exposure (Maltoni and Lefemine, 1974, 1975, Szentesi et al. 1976, Waxweiler et al., 1976, Picciano et al., 1977), the reduction of occupational exposure might considerably contribute to reduction of the hazards of VC exposure. The enormous increase in fetal mortality in the human and the finding of a high incidence of fetal losses after VC exposure of rats and mice indicate that with women in the fertile age a further hazard of VC exposition on intrauterine fetal life must be taken into account.

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