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PRELIMINARY REPORT

# CHICK EMBRYOS AS A PROBE OF THE RELATIVE TOXICITIES OF SOOT SAMPLES FROM A POLYCHLORINATED BIPHENYL-CONTAINING TRANSFORMER

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Chick Embryos as a Probe of the Relative Toxicities of Soot Samples from a Polychlorinated Biphenyl-Containing Transformer. Tumasonis, C. and Kaminsky, L.

The involvement of a polychlorinated biphenyl (PCB)-containing transformer in a fire resulted in widespread contamination of the State Office Building in Binghamton, New York with a soot-like material containing 1 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), 50 ppm 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF), and relatively high concentrations of PCBs and other polychlorinated dibenzofurans and dibenzodioxins. The chick embryo system has been investigated as a rapid and inexpensive method for assessing the relative toxicities of large numbers of soot samples derived from different sites in the building, and as a means of assessing effectiveness of the clean up procedures. Samples were administered to the embryos in an aqueous 0.75% methyl cellulose vehicle via a hole drilled into the shell over the air sac. Soot exhibited the greatest embryo lethality when administered on day 4 of incubation. There was a non-linear dose related embryo lethal response with a marked saturation of embryo lethality between the 0.2 and 2.0 mg soot/egg doses. At 2.0 mg soot/egg administered in 0.1 ml aqueous vehicle approximately 50% of the embryos were dead by day 18 of incubation. No deaths were observed with the same quantities of active carbon or fireplace soot. Increasing volumes of vehicle increased the embryo lethal effects of the soot probably indicating that the non-linear dose response was partially due to a lack of availability of all the administered soot to the inner air sac membrane. At doses of 2.0 mg Binghamton soot/egg some teratological effects were observed in

embryos surviving to day 18 of incubation. The effects included curled toes, anophthalmia, exencephaly and edema and occurred at low frequency and non-reproducibly. 2,3,7,8-TCDD when administered into the eggs at doses of up to 500 ng/egg in the aqueous vehicle and up to 800 ng/egg in corn oil killed 3 out 15 embryos at the highest dose and all embryos above 10 ng/egg in the two vehicles respectively. Further studies are underway to develop the chick embryo system for use in investigating the relative toxicities of different soot samples.

A fire which involved a transformer in the State Office Building in Binghamton, New York resulted in extensive contamination of the building with a soot-like material. The transformer contained a dielectric fluid (pyranol) which comprised 65% Arochlor 1254 (a polychlorinated biphenyl (PCB) mixture), 35% chlorinated benzenes and trace additives.

Soot samples collected from two sites in the building were contaminated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF)(Smith et al., 1981a). Based on pyrolysis studies with PCBs it was predicted that the soot would also have relatively large contamination by other isomers and congeners of the polychlorinated dibenzo dioxins and dibenzofurans (Buser et al., 1978).

Predictions of the toxicity of the soot based on the known concentrations and toxicities of 2,3,7,8-TCDD and 2,3,7,8-TCDF are suspect for a number of reasons: Not all contaminating components of the soot can be determined, the toxicities of all polychlorinated dibenzodioxins and dibenzofurans is not known, the role of the soot matrix was not known (Silkworth et al., 1981) and the potential for synergistic or antagonistic effects of the pollutants on the toxicity of other pollutants is unknown. Thus there was a requirement for a toxicological assessment of the soot where it would be treated as a single compound.

As part of the clean-up procedures in the building, assessments of the variations in toxicity of soot from different sites and at different times as the clean-up proceeds will require toxicologic analysis of numerous samples. A method was required which would provide a rapid analysis of large numbers of samples without enormous

resources of personnel and equipment. These criteria could be met by using chick embryos. In 1966 a group meeting under the auspices of the World Health Organization recommended that the chick embryo not be used for screening drugs for teratogenicity since it is "too sensitive to a wide range of agents and affords no parallel with the anatomical and physiological relationship existing between a pregnant mammal and her conceptus" (World Health Organization, 1967). Subsequently arguments have been raised in favor of the introduction of the use of chick embryos in teratology most notably by Gebhardt (1972) in the Netherlands and Wilson (1978) in the USA. A number of reports have appeared recently in which the chick embryo method has been used to investigate the teratogenicity of methylene chloride, trichlorethane, trichloroethylene, tetrachloroethylene and toluene (Elovaara et al. 1979), DDT (Swartz, 1980), cholinergic insecticides (Misawa et al., 1981) and Lambda-Carrageenan (Monis and Ravasio, 1981). The chick embryo has also recently been used to evaluate the cardiovascular teratogenicity of 2,3,7,8-TCDD (Cheung et al., 1981).

In this paper we report our preliminary investigations on the use of the chick embryo to investigate the relative toxicities of samples of soot collected in the state office building. The main criterion which will be used is embryo lethality rather than teratogenicity in view of the controversy associated with interpretation of teratological effects and the relative ease in assessing embryo lethality.

## METHODS

Fertile White-Leghorn chicken eggs (50-60 g) were obtained from a farm maintained by this Division. After collection the eggs were placed into a humidified (65-67% humidity), forced-draft incubator (Humidaire) and were incubated at 37.5° ± 0.32° C to initiate embryo development.

Contaminated soot was obtained with a vacuum cleaner fitted with clean collection bags from the stairwells of the third and fourth floors of the State Office Building in Binghamton, New York. It was filtered through a wire gauze to remove gross inert contamination. Extracts of the soot were prepared by Soxhlet extraction with benzene for 16 hr. Benzene was removed by heating the solution. 2,3,7,8-TCDD was obtained from Dow, Midland, MI as a solution in toluene. The soot was analyzed as previously reported (Smith et al., 1981b).

For administration of samples a small hole was drilled into the egg shell in the center of the airspace (determined by candling) and samples injected by a syringe (fitted with an 18g needle) onto the inner membrane. The hole was then immediately sealed with paraffin and the eggs incubated. At the end of the incubation period eggs were cracked open and the embryos were removed and evaluated for lethality, gross malformation, and subcutaneous edema. Surviving and non-autolized embryos were individually preserved in 10% neutral buffered formalin.

Studies were performed to determine the optimal day of administration and day of termination, dose response-curve, effect of volume of vehicle, effect of polarity of vehicle and effect of the soot matrix on the toxicity of contaminants. The last study will be performed by comparing the embryo lethality of the soot itself with that

of the benzene extract of the soot. Effect of vehicle polarity was tested using 2,3,7,8-TCDD as the test compound. The vehicle chosen for most of the studies is 0.75% aqueous methyl cellulose. A similar system has recently been demonstrated to be a safe vehicle in teratological studies (Fritz and Decker, 1981). This vehicle has also been used in extensive studies of the toxicity of the soot in guinea pigs (Silkworth et al., 1981).

## RESULTS AND DISCUSSION

Thirty eggs were randomized into three groups of 10 each. On each of days 0, 3 or 4 of incubation one of the groups was inoculated with 2.0 mg soot/egg in 0.75% aqueous methyl cellulose (0.1 ml/egg). One half of each group was opened on day 14 and the remainder on day 18, and the embryos examined for viability. Administration on days 0, 3 and 4 resulted in a total of 17, 38 and 78% embryo deaths respectively. It was thus apparent that administration on day 4 was the most effective for the expression of soot toxicity in this system and all subsequent studies incorporate administration on day 4.

In a series of studies designed to determine the dose response and reproducibility of embryo lethality, eggs were administered 0.02, 0.2 or 2.0 mg soot/egg in aqueous methyl cellulose into the air sac on day 4 of incubation. In each study 15 eggs/group were used. Two control articles, fireplace soot and active carbon, were also inoculated separately on day 4 into the air sac at doses of 2 mg/egg in the aqueous vehicle. In no case did the control articles result in embryo lethality. In two separate experiments 2 mg soot/egg led to 53 and 56% dead embryos indicative of the reproducibility of the system. However, studies repeated at periods over several months showed much greater variability. In one of these experiments only one of the soot-exposed embryo which survived the 18-day incubation exhibited anophthalmia, exencephaly, toe curl and a short upper beak while four other embryos exhibited edema. Toe curl was found for the majority of the chicks surviving up to the 18th day. In the duplicate experiment the only gross teratological effect observed was toe curl.

In Fig. 1 the dose response curve for embryo lethality is shown. There was a marked saturation in embryo lethality between the 0.2

mg/egg and 2.0 mg/egg dose levels. To explain this we hypothesized that the aqueous soot-containing vehicle would form droplets on the inner membrane and that some of the soot, distant from the droplet surface exposed to the membrane, would not be available for xenobiotic release to the embryo. To test this hypothesis we administered to groups of 15 eggs, doses of soot in differing volumes of vehicle. Doses of 1 mg/egg and 0.1 mg/egg were administered in 0.05, 0.10, 0.50 and 1.00 ml aqueous vehicle. No volume effect was noted at the low dose but at the high dose lethality increased with increasing volume until the highest volume where embryo lethality was diminished. Mortalities were 13% at 0.05 ml, 20% at 0.1 ml, 40% at 0.50 ml and 27% at 1.00 ml. It is thus possible that low vehicle volume prevents complete exposure of the soot to the membrane.

In a recent study (Cheung et al., 1981) 2,3,7,8-TCDD was administered into the egg white of fertile chicken eggs in a vehicle of acetone/corn oil at doses of 0.003 to 25 ng/egg on day 0 of incubation. At no dose was there more than 45% lethality. The soot sample used in the current study has been reported to contain approximately 1 ppm of 2,3,7,8-TCDD and 50 ppm 2,3,7,8-TCDF (Smith et al., 1981b). At a dose of 2 mg soot/egg there was thus approximately 2 ng 2,3,7,8-TCDD/egg and 100 ng 2,3,7,8-TCDF/egg.

In an effort to compare the soot embryo lethality with that of one of its similarly administered components, and to determine the effect of the aqueous vehicle relative to a more hydrophobic vehicle 2,3,7,8-TCDD was administered to the eggs in aqueous methyl cellulose or corn oil vehicles. Groups of 15 eggs received 0.1, 1.0, 5.0, 10.0, 100, 250 or 500 ng TCDD/egg in 0.1 ml aqueous methyl cellulose, or 10,

50, 100, 250 or 800 ng TCDD/egg in 0.10 ml corn oil, or 0.1 ml of the vehicles on day 4 of incubation, into the air sac. With the aqueous vehicle only the two highest doses were embryo lethal producing 1 and 3 deaths respectively. In contrast all doses above 10 ng/egg in corn oil killed all the embryos. The corn oil vehicle killed 4 embryos. While this study has not been completed it is apparent that the embryo lethality of the soot probably does not arise from the bound 2,3,7,8-TCDD.

The effect of corn oil itself in killing 27% of the embryos is consistent with a previous report that olive oil (25 $\mu$l/egg) injected into the air sac on day 3 of incubation killed 27% of the embryos by day 14 of incubation (Elovaara et al., 1979). However, in another study (Kitos et al., 1981), corn oil when injected into the yolks of eggs at 50 $\mu$l/egg on day 6 of incubation produced no deaths of embryos by day 18.

In conclusion we have demonstrated that the Binghamton soot produces embryo lethal effects when administered into the air sacs of fertile eggs under conditions where active carbon and fireplace soot have no effect. The lethal effects are dose dependent and also dependent on the volume of administration vehicle. One of the components of the soot, 2,3,7,8-TCDD, is markedly more toxic when administered in corn oil than in an aqueous vehicle and it is possible that the apparent toxicity of the soot is similarly dependent on vehicle polarity. Further studies are underway to develop the chick embryo method for evaluation of relative toxicities of Binghamton soot samples.

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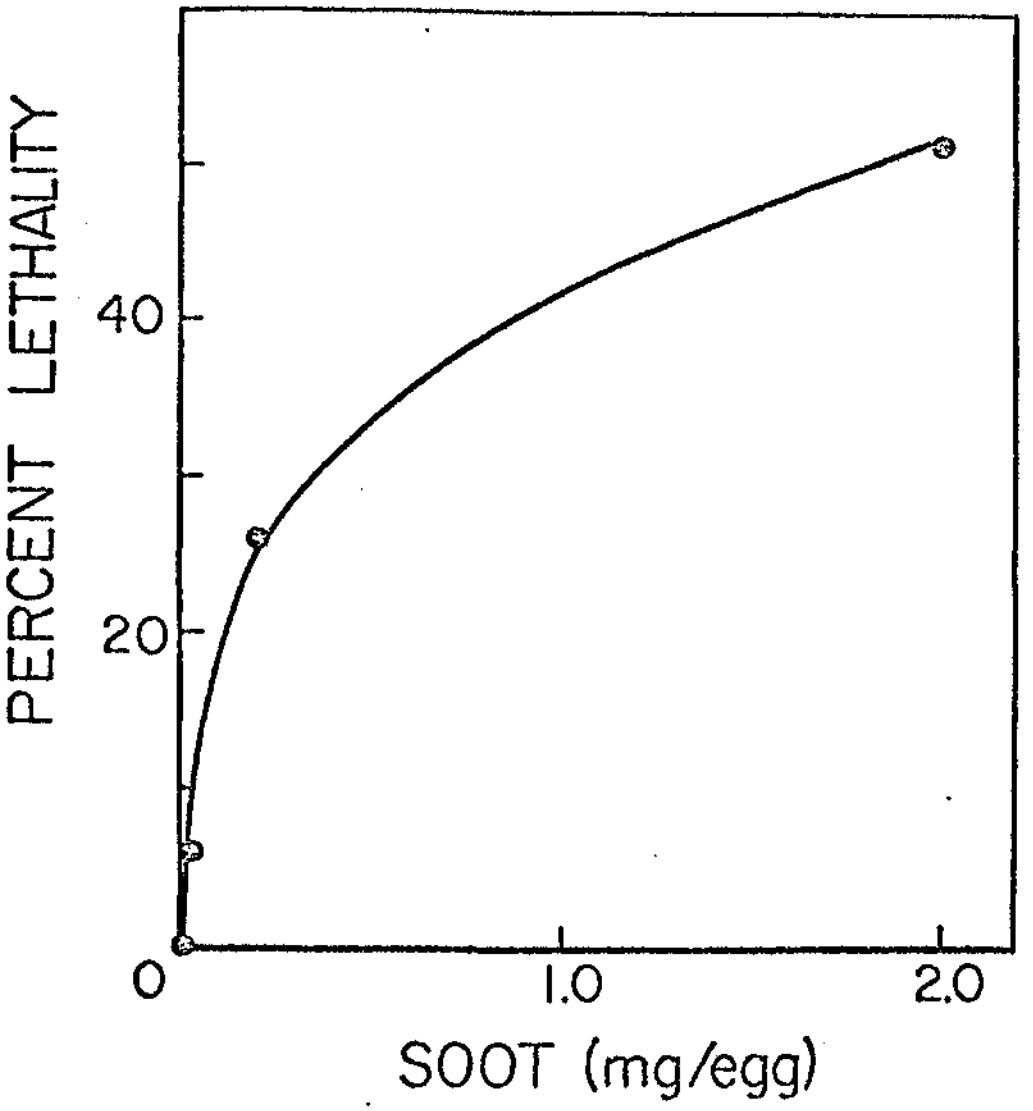
## Figure Legend

Fig. 1. The embryo lethality of Binghamton soot as a function of dose. Soot was administered to fertile chicken eggs in 0.1 ml 0.75% aqueous methyl cellulose through a hole into the air sac on day 4 of incubation. The viability of the embryos was assessed on day 18 of incubation.


PERCENT LETHALITY
40
20
0
1.0
2.0
SOOT (mg/egg)