Tolerance of Three Species of Insects to Prolonged Exposures to Ozone[1]

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It has been postulated that ozone, like ionizing radiation, exerts its major oxidative effects on biological tissue via a free radical mechanism(s). Therefore, ozone has been considered a "radiomimetic" agent (Jaffe 1967; Veninga 1967; Zelac et al. 1971). Jaffe (1967) has shown ozone to be lethal to small laboratory animals exposed to concentrations of 3.6 to 20 ppm within several hours, and to cause an increase in the mortality rate of animals exposed to 0.01 to 0.25 ppm ozone for several weeks.

However, there has been virtually no research concerning the acute and chronic toxicity of ozone to insects. Beard (1955) indicated that prolonged exposures to high levels of ozone (i.e., ca. 0.1 ppm) was lethal to adult house flies, *Musca domestica* L., as well as caused flies to lay fewer eggs per female. Levy et al. (1972) reported increased mortality in adult house flies continuously exposed to ozone for 19 h. In addition, a 15–17% inhibition of egg hatch occurred in *M. domestica* and *Stomoxys calcitrans* (L.) exposed to ozone; however, no such inhibition occurred in *Drosophila melanogaster* Meigen. No mortality resulted when *M. domestica* and *S. calcitrans* larvae and pupae were exposed.

The purpose of this study was to determine if prolonged exposures to high levels of ozone would induce deleterious effects in cockroaches, *Periplaneta americana* (L.), *Nauphoeta cinerea* (Oliver), and the red imported fire ant, *Solenopsis invicta* Buren.

Methods and Materials

Groups of 10–15 ♂ and ♀ adult and nymphal *P. americana, N. cinerea,* and a small colony containing mixed castes (i.e., several thousand major and minor workers, larvae, pupae, and a queen) of the red imported fire ant, *S. invicta,* were continuously exposed to high levels of ozone generated by ultraviolet light (UV) for 22–26 days in a hyperbaric chamber.

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Procedures and equipment for insect ozonation have been previously described by Levy et al. (1972). Ozone output within the hyperbaric chamber was maximal at 140 V. The ozone atmosphere was presumed to reach a concentration of 0.3 ppm or greater, based on the Mast Meter determinations of Zelac et al. (1971), who used the same chamber and UV lamp.

Cockroaches and fire ants were exposed in cardboard canisters covered with finely meshed cloth, and in a small plexiglass ant nest (Wilson 1962[4]), placed within a plastic tub, respectively. The sides of the tub were dusted with inert talc to prevent the ants from escaping. Ten presumably fertile queens (collected after a mating flight) were also exposed individually on moistened paper toweling in jars for short periods of 1–3 days, or for extended periods of up to 10 days, with 9 minor workers and 1 major worker.

Temperature within the chamber and room was relatively constant at 23.9°C. Sufficient water (moisture) was maintained within each insect-holding cage by the addition of wet cotton for the cockroaches, and by adjusting a water-filled syringe attached to the plexiglass nest for the fire ants. Food was supplied as needed. Controls were treated in a similar manner.

The hyperbaric chamber was saturated with ozone for several hours prior to the introduction of the insects, to build up the active concentrations within the chamber and, therefore, avoid reduced concentrations due to absorption and reactivity with the chamber and cage materials. The chamber was opened briefly several times during the course of the experiment, to make observations as well as to check the moisture and food supply.

Results and Discussion

Ozone Effects on Cockroaches

Prolonged exposures to high concentrations of ozone (i.e., 0.3 ppm—Zelac et al. 1972) produced no significant increase in adult and nymphal mortality during the exposure period. In addition, observations for several weeks postexposure indicated no ozone-induced increase in mortality vs. controls. Several females carrying oothecae were exposed with each test group. No ozone-induced inhibition of egg hatch or nymphal to adult development was observed.

No differences in ozone sensitivity between sexes or between species of cockroaches was observed. These results were surprising, since ozone has been reported to be a "radiomimetic" agent (Zelac et al. 1972), and Cromroy et al. (1971) have reported a wide difference between the LD_{50} radiosensitivity of adult *P. americana* and *N. cinerea*.

Observations for 6 weeks postexposure revealed no gross abnormalities in physical characteristics, color, or behavior.

Ozone Effects on Fire Ants

No differences were detected between workers (major and minor) and larvae and pupae during exposure or several weeks postexposure. Prolonged exposure (10 days) did not increase the mortality of queens vs. controls. Eggs were obtained from several of the exposed queens.

Initial exposure of the fire ant colony to the ozone-saturated chamber appeared to stimulate the workers, many of which were outside the plexiglass nest, to migrate inside the nest, possibly indicating a negative ozone response. However, 6 weeks of postexposure observations indicated that ozone did not alter general behavior, or inhibit normal colony functions such as feeding and brood tending. No inhibition of molting or metamorphosis, or abnormalities in color or physical characteristics, occurred.

In another experiment, several groups of exposed (26 days) and nonexposed red imported fire ant workers were allowed to feed on various concentrations of mirex bait, according to procedures described by Levy et al. (1973), to determine if prolonged exposures to high concentrations of ozone would increase or decrease their tolerance to this insecticide. Results indicated no significant differences in mortality between the exposed and nonexposed groups.

In general, results indicated that prolonged exposure to high concentrations of ozone did not produce any noticeable deleterious effects to adult or immature stages of *P. americana*, *N. cinerea*, and *S. invicta*. It appeared that these 3 species of insects possessed a greater tolerance to the toxic effects of ozone than house flies (Beard 1965; Levy et al. 1972) or mammals (Jaffe 1967). It would seem that higher concentrations of ozone than we have generated are required to induce lethality and inhibition of life cycle processes, possibly due to a negative spiracular response (Levy et al. 1972) and inducement of insect "autotolerance" (Veninga 1967).

[4] Illustration only; description not given.

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