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Woodlice Exposed to Pollutant Gases

Joseph J. White

Department of Biology, A & T State University, Greensboro, NC 27411

The considerable literature which has developed on the effects of pollutant gases on plants shows that, in general, plants are vulnerable to even low gas concentrations (MACDOWALL 1965; TINGEY et al. 1971; CONSTANTINIDOU et al. 1976; SWIEBODA & KALEMBA 1978). On the other hand, studies involving invertebrate animals indicate a considerable variability in their response to pollutant gases. High concentrations of SO_2 are necessary when SO_2 is used as a fumigant insecticide (SHEPARD et al. 1937; GOUGH 1949; BUSVINE 1942). Concentrations of SO_2 up to 9,068 ppm were necessary to kill some insect pests. Yet SO_2 concentration as low as 0.45 ppm produced a 50% mortality in the bark-living mite Humerobates rostrolamellatus in two days (LEBRUM et al. 1978). Sulfur dioxide in concentrations ranging from 0.4 to 0.7 ppm adversely affected Drosophila melanogaster (GINEVAN & LANE 1978). The female parasitic wasp Bracon hebetor was exposed to 3 ppm SO_2 for up to five hours. During the twenty days which followed, the reproductive performance of these wasps was assayed. Lifespan and egg production of the treated females were not different from the controls, and hatchability was not consistently affected (PETTERS & METTUS) 1982).

Because of the unique digestive and respiratory habits of woodlice and the importance of woodlice as litter decomposers in terrestrial ecosystems, a decision was made to assess the effects of sulfur dioxide (SO_2), nitrogen dioxide (NO_2), carbon monoxide (CO), and methane (CH_4) on these organisms directly through the air they breathe and indirectly through the food they eat. Woodlice have a limited need for free oxygen and can survive up to nine weeks in closed containers (HEELEY 1941a). Their respiratory structures include pleopods which are located beneath the abdomen. Those species of woodlice that have their pleopods modified by pseudotracheae are better able to respire in dry air. Among the gases chosen from this investigation, it is known that SO_2 in water forms sulfurous acid (H_2SO_3), which in small quantities is oxidized to sulfuric acid on exposure to air. Nitrogen dioxide (NO_2) is soluble in water. It combines with water to form a mixture of nitrous and nitric acids. Carbon monoxide (CO) acts as a poison on the mitochondria and cytochrome enzyme systems. Methane (CH_4) is quite inert and resists the action of most reagents. Woodlice eat moist litter which contains cellulose. Thus, woodlice might take in pollutants not only through respiratory routes, but also through the food they eat. In addition to

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investigating the effects of single gases on woodlice, the effects of all possible gas mixtures were studied in a series of 2x2 factorial experiments to test for deleterious interaction effects.

MATERIALS AND METHODS

The woodlice, Tracheoniscus rathkei, used in this experiment were obtained in Guilford County, North Carolina, U.S.A. The gases (NO_2, CO, SO_2 and CH_4) were obtained from Airco Industrial Gases, a division of Airco Inc., a national gas supply company. The gas concentrations were mixed and warrantied by the company. Woodlice observation chambers consisted of two glass spherical O-ring joints with Teflon stopcocks in each end. Between the two joints was placed a Teflon-coated O-ring, and the two joints were held together by a pinch clamp. The chambers were made of these materials in order to preclude any reaction between the chambers and the gases. They were obtained from University Research Glassware of Carrboro, North Carolina. Because proper humidity is essential for woodlice survival, moistened six gram clay flower pot shards were used. Preliminary investigation showed that this size shard maintained the proper humidity during the eight day experiments. Dampened 1 gram pieces of Poplar bark were supplied for food.

Woodlice were tested by exposing them to three concentrations of a given gas and simultaneously running a natural air control (Table 1). Thirty-six observation chambers (nine replicates of each treatment) with ten woodlice each, were divided into control (9 chambers) and 3 experimental groups (9 chambers each) with concentration differences spanning 5-10 ppm.

The set-up procedure involved placing 10 woodlice in each chamber, sealing the joint with a pinch clamp, and by using a vacuum pump, creating a sufficient vacuum in the chamber to reduce its pressure to 26 cm of Hg below ambient (measured on a Hg manometer). Using the gas cylinder regulator and a Telfon tube connected to the gas cylinder and the observation chamber, the pollutant gas was introduced to the chamber. Then the combination of pollutant gas and ambient air was vacuumed and the chamber refilled with pollutant gas. This procedure was repeated four times to assure the removal of all ambient air. The advantage of this procedure is that it prevents the woodlice from being exposed to complete vacuum at any time. Control woodlice received the same treatment as experimentals except ambient air rather than gas was drawn into the chambers.

The 27 experimental chambers and the 9 control chambers were placed in a dark enclosure. After four days the surviving woodlice were observed for activity and counted. Woodlice kept in the dark become active when exposed to light (TORREY & HAYS 1914). The gas in each chamber was then replaced by fresh pollutant gas, or fresh air in the case of controls. At the end of a second four days the surviving woodlice were again observed for activity, counted, and removed from the observation chambers. Effects of pollutant gases were evaluated by their effect on woodlice survivorship at the end of 8 days.

To assess the possible deleterious interaction effects of the pollutant gases, 2x2 factorial design experiments were set up with gas combinations obtained from Airco. Using the same procedure as described above for introducing the woodlice and the single gases into the observation chambers, the woodlice were exposed to the six gas combinations, while a natural air control was observed. Effects were again evaluated on the basis of survivorship after 8 days of exposure.

RESULTS

The results of exposing the woodlouse, Tracheoniscus rathkei, to various concentrations of single pollutant gases for given time periods are shown in Table 1. The percentage of surviving woodlice at each gas concentration at the conclusion of two consecutive observation periods - four and eight days - was the assay for pollutant gas effects. Table 1 shows that mortality among experimental animals exposed to a given gas was not significantly greater than among controls. This suggests that woodlice are relatively resistant to single pollutant gases at the levels tested.

Table 1 Percent surviving woodlice at 4 days and 8 days.

Gas			Conc. 1	Conc. 2	Conc. 3	Conrol
NO_2			18.1ppm	24.2ppm	30.1ppm	control
	4 days	$\bar{X}$%	94.4	90.0	88.8	84.4
		SD	7.3	15.8	13.6	18.8
	8 days	$\bar{X}$%	75.3	63.3	65.5	64.4
		SD	15.1	23.3	25.5	28.3
SO_2			18.5ppm	23.4ppm	25.8ppm	control
	4 days	$\bar{X}$%	82.2	86.6	85.5	84.4
		SD	10.0	12.2	19.4	10.1
	8 days	$\bar{X}$%	65.5	53.3	71.1	63.3
		SD	14.2	26.9	27.6	25.5
CH_4			26.1ppm	30.0ppm	34.5ppm	control
	4 days	$\bar{X}$%	82.2	87.7	85.5	84.4
		SD	12.0	9.7	8.8	11.3
	8 days	$\bar{X}$%	72.2	72.2	63.3	78.8
		SD	9.7	13.9	10.0	10.5
CO			9.0ppm	14.5ppm	23.3ppm	control
	4 days	$\bar{X}$%	90.0	88.8	87.7	88.8
		SD	11.2	6.0	9.7	15.4
	8 days	$\bar{X}$%	73.3	76.7	71.1	67.7
		SD	12.2	7.71	14.5	15.6

Finally, in assaying the effect of the gases in combination, it was noted that a significant deleterious interaction was seen in one of the six gas combinations used. Table 2 shows that NO_2 in combination with SO_2 caused a significant decrease in survival which could not be attributed to either of the gases acting singly. NO_2 in combination with SO_2 seems to have a deleterious synergistic effect on woodlice.

Table 2. Descriptive statistics and ANOVA for interaction experiment involving NO_2 & SO_2.

		NO_2 0.0ppm		NO_2 30ppm	
		Control		Experimental	
SO_2	0.0ppm	$\bar{x}$ = 92.2	sd = 9.7	$\bar{x}$ = 85.5	sd = 14.2
		Experimental		Combination	
	30ppm	$\bar{x}$ = 92.8	sd = 9.7	$\bar{x}$ = 78.8	sd = 6.0

	Source of Variation	df	Mean Square	F ratio
ANOVA table	NO_2	1	2.8	0.04
	SO_2	1	25	0.36
	NO_2 & SO_2	1	625	0.91*$P<0.01$
	Error	32	70.1	

DISCUSSION

In theory, woodlice should be ideal organisms to serve as biomonitors of air pollution since these pollutants can enter their bodies through both oral and respiratory routes. That their resistance is so high and their averaging time is so long renders them less than effective for such uses. When Table 3, which shows the primary and secondary ambient air quality standards set up by the U.S. Environmental Protection Agency (1971), is compared to Table 1, which shows the survivorship of woodlice under the experimental conditions, it becomes apparent why woodlice might not be useful as monitors of pollutants. Even SO_2, which is very soluble in water and very troublesome to some plants and animals, showed no significant effect on woodlice. The effect of SO_2 appears to depend on the species of organism involved and on whether the SO_2 is in the gaseous state only, or is combined with particulate matter (BERRY et al. 1974).

Table 3. U.S. National Ambient air quality standards.

Contaminant	Averaging time	Primary standard µg/m³	Primary standard ppm	Secondary standard µg/m³	Secondary standard ppm
Total suspended particulate matter (TSP)	Annual mean (geometric)	75		60	
	24 h[a]	260		150	
Sulfur dioxide	Annual mean (arithmetic)	80	0.03		
	24 h[a]	365	0.14		
	3 h[a]			1300	0.5
Carbon monoxide	8 h[a]	10 mg/m³	9	10 mg/m³	9
	1 h[a]	40 mg/m³	35	40 mg/m³	35
Photochemical oxidants (ozone)	1 h[a]	240	0.12	240	0.12
Nitrogen dioxide	Annual mean (arithmetic)	100	0.05	100	0.05
Hydrocarbons (nonmethane)	3 h[a] (6 to 9 A.M.)	160	0.24	160	0.24

[a] Not to be exceeded more than once per year.

The role played by synergism in the promulgation of pollution is well established. It is seen in photochemical smog, a product of the synergistic interaction between the various oxides of nitrogen and hydrocarbons. Synergism is also seen where fine particles (less than 0.002 mm) of soot or smoke absorb hydrocarbons and metal atoms and carry these into the lungs where they become trapped. Synergism is observed in the result of combining air pollution and smoking, it being noted that the general effect of air pollution is more deleterious on the health of smokers than on nonsmokers.

In this investigation a synergistic interaction between NO_2 and SO_2 was demonstrated by the effect this combination had on woodlice. The mortality rate in the experimental chambers that contained this combination was significantly higher than in those chambers that contained single gases.

Two general conclusions result from this investigation. One is that while some higher organisms may be adversely affected by low concentrations of a single pollutant gas, toxicity is experienced by woodlice only if the gases are combined and in high concentration. Since except at the point source, pollutant gases may not occur in high concentration, woodlice may better monitor those pollutants which combine in various ways to produce synergistic interactions. The second general conclusion is that the standards established for the six regulated pollutants and shown in Table 3 may be adequate in those instances where the pollutants occur singly and in the concentrations and with the averaging times listed. However, it is more likely that several pollutants will be present at one time thus providing several avenues for combinations and interactions to occur. This leads to the conclusion that standards need to be written which take such combinations into consideration and that the determination of the deleterious effects of pollutants will remain incomplete until such combinations are considered.

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