Influence of Temperature on Toxicity of Propylene Oxide at Low Pressure Against *Tribolium castaneum*

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Toxicity of propylene oxide (PPO) at low pressure against the most common stored-product insect, *Tribolium castaneum* (Herbst), over a short exposure time, was tested at three different temperatures (16°, 22° and 30°C). Toxicities of PPO at 100 mm Hg were strongly influenced by ambient temperature. LD_{50} and LD_{99} toxicities ranged from 4.7 to 28.9 mg l^{-1} and from 10.5 to 72.6 mg l^{-1} respectively, showing that susceptibility was positively correlated to the temperature. The LD_{99} values for all life stages (except the larval stage) were significantly lower at 30° than those at 16° and 22°C. However, the LD_{99} values for all life stages (except the pupal stage) at 16° were not significantly different from those at 22°C. A concentration × time (Ct) product of 291, 171 and 98 mg h/*l* was required to obtain complete mortality (99%) of *T. castaneum* at 16°, 22° and 30°C, respectively. Thus, the efficacy of PPO at 100 mm Hg to all life stages of *T. castaneum* also decreased as the temperature decreased from 30° to 16°C.

KEY WORDS: Propylene oxide; fumigant; toxicity; temperature; *Tribolium castaneum*.

INTRODUCTION

The fumigant methyl bromide has been used for many years to treat the commodities that have become infested with stored-product insect pests. However, methyl bromide is being withdrawn from use because it has been listed as an ozone-depleting substance by the United Nations Environmental Programme. Under the terms of the Montreal Protocol, it is due to be phased out by 2005 in developed countries. Therefore, worldwide efforts have focused on finding suitable alternatives for use as postharvest commodity treatments. Recent, preliminary reports on insect toxicity indicate that propylene oxide (PPO) might be an effective replacement for methyl bromide in some postharvest situations (3,7). As a fumigant, PPO has reduced environmental risks compared with methyl bromide. It is not an ozone depleter and it degrades into nontoxic propylene glycol in the soil and in the human stomach.

Propylene oxide is commonly used as an insecticidal fumigant and as a sterilant to reduce bacteria, mould and yeast contamination on processed spices, cocoa and processed nutmeats (except peanuts). It is a liquid fumigant under normal temperature pressure, with a boiling point at 35°C and a noticeable ether odor (21). It is a safe fumigant for use on food as a sterilant to reduce microflora and microfauna because it is quickly converted to non-toxic glycols in the human stomach. The human health and environmental effects of PPO have been reviewed in Meylan *et al.* (13). A disadvantage of PPO is that it is

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flammable from 3% to 37% in air, and therefore to avoid flammability it should be applied under low pressure or in a CO_2-enriched atmosphere. PPO has also been considered for rapid disinfestation of durable stored products as a replacement for methyl bromide (11,16). Isikber *et al.* (10) and Navarro *et al.* (16) compared the relative effectiveness of PPO alone and in combination with low pressure or CO_2 by determining the dosages required for critical mortalities of all life stages of *T. castaneum* at a short exposure time of 4 h. Their study revealed that 100 mm Hg and 92% CO_2 had a synergistic effect on the toxicity of PPO to *T. castaneum* and the combination of PPO with low pressure or CO_2 can provide a potential alternative to MB.

In developing commercial fumigation schedules, it is necessary to know the efficacy of the fumigant against a wide range of storage pests under a variety of fumigation conditions, and the sorption kinetics of the fumigant on the treated commodity, since the rate of sorption affects the insecticidal efficacy of the fumigant. Zettler *et al.* (22) reported that there was considerable gas loss due to precipitation of the fumigant on the chamber wall because of the difficulty in maintaining the fumigant in its gaseous state at temperatures below its boiling point. They reported that this situation contributes to PPO's overall sorption characteristics and renders the use of fumigants at low temperatures problematic. Although the label authorizes the use of PPO as an insecticidal fumigant for control of stored-product insects, only limited information is available on the efficacy of PPO in combination with low pressure against stored-product insects at different temperatures. Therefore, the objective of this paper was to determine the influence of the temperature on toxicity of PPO at low pressure against the most common stored-product insect, *T. castaneum,* over a short exposure time.

MATERIALS AND METHODS

Test insects Tests were carried out on all life stages of red flour beetle, *T. castaneum*. All stages were obtained from cultures reared at 26±1°C and 70±5% relative humidity (r.h.) on a diet of ground wheat and yeast using standard culture techniques (4). Cultures were started from eggs obtained by sieving from 750 ml oviposition jars containing adults in 400 g of wheat flour. Eggs were separated from oviposition jars by sieving daily. Eggs for exposure to treatments were transferred into 'pits' drilled into Perspex exposure slides, each slide containing 50 pits. When filled, the slides were covered with a cover glass to retain the eggs (15). Two slides containing 100 eggs aged 1–2 days were exposed to each treatment. Larvae were removed from culture jars 12 days after oviposition and exposed to the treatments. Two-day-old pupae were obtained by daily separation from culture jars and held in wheat flour for 24 h before the exposure. Newly emerged adults were held in pre-exposure jars containing wheat flour, and exposed to treatments 7–10 days after emergence.

Fumigation chambers Test chambers consisted of 2.64-*l* desiccators, each capped with a ground-glass stopper equipped with entry and exit tubing. A magnetic stirrer placed in the bottom well beneath a wire-mesh disc served to mix the air with the fumigant. Two pieces of rubber tubing, 5 cm long and 6.2 mm ID, were attached to the tubing and sealed with pinch-clamps. The desiccators were sealed with silicone vacuum grease. The fumigant was >99% pure liquid PPO that was withdrawn from a sealed vial fitted with a rubber septum, using a gas-tight syringe.

Dosing and fumigation procedures Toxicity tests were carried out at three different temperatures: 16°, 22° and 30°C. PPO was introduced as a liquid into the desiccators using a 50 or 250 μl micro-syringe. Pressure in each desiccator was measured using a 0 to 800 mm Hg Celesco-model SE-2000 (Tedea Ltd., Herzliyya, Israel) vacuum gauge. The 100-mm-Hg measure referred to herein is absolute pressure, with 760 mm Hg considered as atmospheric pressure. Prior to each test, 30 larvae, pupae or adults were confined, separately, inside 3-cm-diam $\times$ 8-cm-long wire-mesh cages. For eggs, two exposure slides each holding 50 eggs, were used per fumigation.

For fumigations at low pressure, the insects were first placed in the desiccators and then, prior to introduction of the required PPO concentration, 100 mm Hg was obtained by evacuating air. PPO at 100 mm Hg was tested at the following dosages, depending on the life stages of the tested insect. At 16°C, dosages (in mg l^{-1}) were: 5–20 for adults, 20–70 for pupae, 10–50 for eggs, and 5–20 for larvae; at 22°C: 7.5–20 for adults, 10–40 for pupae, 10–40 for eggs, and 10–20 for larvae; and at 30°C: 5–20 for adults, 5–30 for pupae, 5–30 for eggs, and 2.5–15 for larvae. Each test, of untreated control insects exposed to atmospheric conditions, was replicated at least twice. A 4-h exposure time was used for all experiments. The gas mixtures in the desiccators were stirred for at least 20 min. For all fumigations, r.h. and temperature were maintained at 65$\pm$5% at atmospheric pressure using saturated $MgNO_3$ salt and 30$\pm$1°C, respectively. However, the r.h. decreased to 50$\pm$5% at the initial stage of the treatment when 100 mm Hg was applied, but it returned to 65$\pm$5% within 0.5-h after the treatment. Pressure inside the desiccators was checked after injecting the PPO and at the end of each test. Relative humidity during fumigations was also measured, by placing small mechanical hygrometers within the desiccators.

Fumigant analysis Concentrations of PPO in each desiccator were checked at the beginning and end of the 4-h exposure period by withdrawing a 15-μl gas sample from the exposure chamber, using a 50-μl gastight syringe. The concentration of PPO was measured using a Shimadzu 17A gas chromatograph (Agntech Ltd., Tel Aviv, Israel) fitted with a flame ionization detector and an Econo-Cap EC^{TM}–Wax capillary column (30 m length $\times$ 0.25 mm ID $\times$ 0.25 μm film thickness) (Alltech, Deerfield, IL, USA) run at 170°C isothermal. During the operation, gas flow rates were 30, 50 and 500 ml min^{-1} for helium, hydrogen and air, respectively. Temperatures were 170°, 250° and 260°C for column oven, injector port and detector, respectively. Under these conditions, the retention time of PPO was *ca* 2.65 min.

Data processing and analysis After each treatment, larvae, pupae and adults were transferred to 200-ml jars containing standard diets and were held at 26$\pm$1°C and 70$\pm$5% r.h. until examined for mortality. The eggs in their Perspex slides were held under the same conditions until the oviposition sites were examined for egg hatch. Mortality counts for adults were done 4–5 days after exposure; for larvae they were based on those insects that had failed to pupate 9 days after exposure; pupal mortality was based on those pupae that failed to produce adults 9 days after exposure; and egg hatch was counted 7 days after treatment. Zero dose control and dose-mortality responses were subjected to probit analysis by the POLO-PC computer program (12) to determine the LD_{50}, LD_{99} and their respective 95% confidence intervals. Differences in toxicity were considered significant when 99% confidence intervals did not overlap. The slopes and intercepts of concentration-mortality regressions for each tested insect were compared with the POLO-PC maximum-

likelihood procedures (12). The concentration × time (Ct) products required to obtain 50% and 99% mortality of all insect stages of each insect were calculated using the LD_{50} and LD_{99} concentrations derived from probit analyses.

RESULTS

Table 1 shows probit mortality regression data for PPO at 100 mm Hg against all life stages of *T. castaneum* at three different temperatures. There was a remarkable difference in susceptibility to PPO at 100 mm Hg between the life stages. While toxicities (LD_{99}) for the adults and larvae at 16°, 22° and 30°C ranged from 10.5 to 19.7 mg l^{-1}, LD_{99} for the eggs and pupae at these three temperatures ranged from 18.9 to 72.6 mg l^{-1}, which reflects decreasing susceptibilities on the order of adult, larva, egg and pupa. Thus, the egg and pupal stages of *T. castaneum* were more tolerant to PPO at 100 mm Hg than the larvae and adults at each temperature. Calculations of Ct products reveal a high Ct product of 291, 171 and 98 mg h/*l* required to obtain 99% kill of pupae at 16°, 22° and 30°C, respectively, whereas for adults only the Ct product of 76, 63 and 42 mg h/*l* was required for 99% kill at 16°, 22° and 30°C, respectively (Fig. 1).

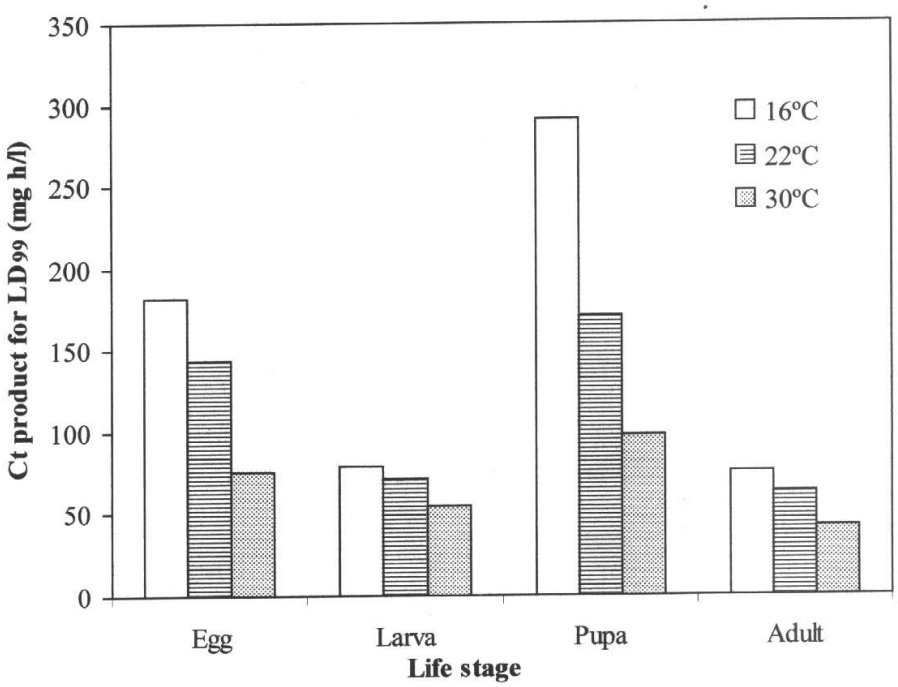

Fig. 1. Ct products (mg h/*l*) required for LD_{99} values at three different temperatures after 4 h laboratory fumigations of propylene oxide at 100 mm Hg against all life stages of *Tribolium castaneum.*

Toxicities of PPO at 100 Hg were strongly influenced by ambient temperature (Table 1). The LD_{50} for all life stages ranged from 4.7 to 28.9 mg l^{-1}, reflecting decreasing

TABLE 1. Probit analysis data for propylene oxide at low pressure of 100 mm Hg for all life stages of *Tribolium castaneum* resulting from 4-h laboratory fumigations at three different temperatures

Stage	Temperature (°C)	n[z]	Slope±S.E.	LD_{50} (mg l^{-1})	LD_{99} (mg l^{-1})	H[x]
Egg	16	800	11.8±1.82	28.9 (26.79-30.36)[y]	45.5 (41.76-52.85)[y]	0.77
	22	1000	11.8±1.29	22.8 (20.77-24.33)	35.9 (31.80-46.56)	1.26
	30	1000	6.8±1.26	8.5 (7.34-9.30)	18.9 (16.04-26.44)	0.31
Larva	16	240	5.5±0.64	7.3 (6.38-8.11)	19.7 (16.47-25.70)	0.09
	22	240	6.5±0.69	7.8 (7.06-8.57)	17.8 (15.33-22.19)	0.81
	30	300	5.0±0.49	4.7 (4.22-5.13)	13.6 (11.41-17.5)	0.84
Pupa	16	240	5.6±0.61	27.6 (24.62-30.51)	72.6 (61.49-92.43)	0.59
	22	240	5.1±0.54	15.1 (13.26-16.84)	42.8 (36.32-54.11)	0.59
	30	240	5.3±0.55	8.8 (7.94-9.73)	24.5 (20.21-32.24)	0.53
Adult	16	280	7.1±0.80	8.8 (8.08-9.60)	18.9 (16.35-23.40)	0.37
	22	280	10.0±1.35	9.2 (8.66-9.72)	15.7 (13.90-19.27)	0.02
	30	280	12.3±1.46	6.8 (6.45-7.16)	10.5 (9.63-12.02)	0.03

[z] Number treated, excluding controls.

[y] Numbers in parentheses give the 95% fiducial limits.

[x] Heterogeneity factor, chi-square/degrees of freedom (chi-square is significant, $P<0.05$).

susceptibilities on the order of 16°, 22° and 30°C. Similarly, LD_{99} ranged from 10.5 to 72.6 mg l^{-1}, reflecting decreasing susceptibilities on the order of 16°, 22° and 30°C. The LD_{99} at 16°, 22° and 30°C for the egg, larva, pupa and adult were decreased by 21%, 9.6%, 41% and 20% as the temperature increased from 16° to 22°C and by 58.5%, 30.9%, 65.8% and 44.4% from 16° to 30°C (Table 1). Based on no overlap in 95% fiducial limit (FL) values, the LD_{99} for all life stages (except larval stage) was significantly lower at 30°C than those at 16° and 22°C. However, LD_{99} values for all life stages (except pupal stage) at 16°C were not significantly different from those at 22°C, since the 95% FL values overlapped. Hypothesis tests for parallelism and equality (18) indicated that regression lines for the egg, larva and pupa were parallel (γ^2 = 5.3, 3.2 and 0.26, respectively) and unequal (γ^2 = 364.1, 58.6 and 147.9, respectively). Parallel probit lines show that the egg, larva and pupa of *T. castaneum* responded to PPO at three temperatures in the same manner. However, the regression lines for the adult were neither parallel (γ^2 = 11.6) nor equal (χ^2 = 65.8). Non-parallel probit lines indicate that the adult of *T. castaneum* did not respond to PPO at three temperatures in the same manner.

Concentrations of PPO (mg l^{-1}) in a 2.64-*l* fumigation chamber at the beginning of fumigation and after 4 h fumigation of the pupae in rearing medium, with 30 mg l^{-1} at three different temperatures, are shown in Figure 1. At all temperatures, it became clear that there was a decrease in concentrations of PPO after 4 h of exposure. The drop in concentrations during 4-h fumigations at 16° and 22°C was, respectively, 66.6% and 48.2% of the initial dosage applied, which was much higher than the 21.4% drop at 30°C. This indicates that there was a higher gas loss throughout the 4-h fumigation at lower temperatures (16° and 22°C) than at the high temperature (30°C). Reducing the temperature from 30° to 16°C resulted in significant sorption that played an important role in achieving complete insect control during fumigation.

Ct products (mg h/*l*) required for LD_{99} values at three different temperatures resulting from 4-h laboratory fumigations of PPO at 100 mm Hg against all life stages of *T. castaneum* are presented in Figure 1. A Ct product of 291, 171 and 98 mg h/*l* was required to obtain complete mortality (99%) for *T. castaneum* at 16°, 22° and 30°C, respectively. An increase in temperature from 16° to 22°C or from 16° to 30°C resulted in a decrease of the Ct by 41% and 66%, respectively, to obtain complete mortality of all life stages of *T. castaneum*. The increase from 16° to 30°C had the greater effect on the Ct products compared with the increase from 16° to 22°C.

DISCUSSION

Temperature affects the physical and chemical properties of gases. It is therefore important to carry out a process, such as fumigation, at a known or fixed temperature. The volatility of a fumigant increases with rising temperature (20). The temperature is highly important in determining the effectiveness of a fumigant, owing to the fact that the rate of respiration is closely correlated with the rate at which insects absorb a fumigant. In general, the toxicity of a fumigant increases with an increase in temperature (20). Several studies have recently demonstrated the insecticidal properties of PPO using the processed food sterilization protocol under vacuum (3,7). However, further developmental work is warranted in order to define the effects of PPO on the insect toxicity spectra, so that accurate PPO treatment schedules for postharvest insect pests can be determined.

Toxicities of PPO at 100 Hg were markedly influenced by the ambient temperature. As the temperature decreased from 30° to 16°C, the efficacy of PPO at 100 mm Hg to all life stages of *T. castaneum* also decreased. Our observation agrees with the results of previous studies of the effect of low temperatures on reducing the response of insects to methyl bromide (1,5,6,19). This reduced response may be explained, in part, by the reduction in the size of the opening of the insect spiracles (2,14) and also by the reduced rate of respiration of insects at lower temperatures.

When the temperature was reduced from 30° to 22° or 16°C, there was considerable gas loss in PPO fumigation, presumably due to sorption by the rearing medium containing the insect bioassays and precipitation of PPO on chamber walls because of the increase in condensation of PPO at treatment temperatures well below its boiling point. Similarly, Zettler *et al.* (22) reported that because of the difficulty in maintaining the fumigant in its gaseous state at temperatures below its boiling point, there is considerable gas loss due to precipitation of the fumigant onto chamber walls. This situation could also explain the low insect toxicity of PPO at low temperatures. Therefore, it appears that careful dosing is required to achieve complete insect control during PPO fumigation.

The egg and pupal stages of stored-product insects are generally more tolerant than larvae or adults to carbon dioxide and to the fumigants methyl bromide (8), phosphine (3,9), and carbonyl sulfide (17). Our data support this generalization for each temperature. Navarro *et al.* (16) obtained complete mortality of all life stages of red flour beetle, *T. castaneum*, at 30°C with a concentration of 24.7 mg l^{-1} at 4 h (C × t = 98.8 mg h/*l*). Our findings at 30°C are similar to those reported by Navarro *et al.* (16).

Isikber *et al.* (11) and Navarro *et al.* (16) reported that the combination of PPO with low pressure or CO_2 can provide a potential alternative to methyl bromide for quarantine treatment of commodities where rapid disinfestation techniques and a high level of insect mortality are essential. In developing commercial fumigation schedules for PPO at low pressure, our toxicity data for PPO at various temperatures could contribute to the formation of a database for PPO fumigations, thus enabling fumigation operators to calculate the dosage required to obtain complete control of insect infestations.

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