Toxicity of Propylene Oxide at Low Pressure Against Life Stages of Four Species of Stored Product Insects

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ABSTRACT The relative toxicity of propylene oxide (PPO) at a low pressure of 100 mm Hg to four species of stored product insect at 30°C over a 4-h exposure period was investigated. PPO at 100 mm Hg was toxic to all four species tested: *Tribolium castaneum* (Herbst), *Plodia interpunctella* (Hübner), *Ephestia cautella* (Wlk.), and *Oryzaephilus surinamensis* (L.). There were differences in susceptibility between the life stages of the tested insect species. Mortality tests on all life stages of the insects resulted in LD_{99} values ranging from 4.7 to 26.1 mg/liter. The pupal stage of *E. cautella*, *O. surinamensis*, and *T. castaneum* was the most tolerant stage with LD_{99} values of 14.4, 26.1, and 25.7 mg/liter, respectively. For *P. interpunctella*, the egg stage was most tolerant, with a LD_{99} value of 15.3 mg/liter. Generally, PPO at 100 mm Hg was more toxic to *P. interpunctella* and *E. cautella* than to *O. surinamensis* and *T. castaneum*. A 99% mortality of all life stages of the tested species was achieved at a concentrations × time product of 104.4 mg h/liter. These findings indicate that a combination of PPO with low pressure can render the fumigant a potential alternative to methyl bromide for rapid disinfestation of commodities.

KEY WORDS propylene oxide, *Tribolium castaneum*, *Plodia interpunctella*, *Ephestia cautella*, *Oryzaephlius surinamensis*

ALTHOUGH MANY DIFFERENT TOXIC gases have been used for disinfestation of stored products such as food grains, grain products, nuts, and animal feed for >100 yr, during the last two decades methyl bromide (MB) and phosphine (PH_3) have, in general, replaced other fumigants. Now MB is being phased out under the Montreal Protocol because of its ozone depletion potential (UNEP 1995). Consequently, for the disinfestation of durable stored products, PH_3 remains almost the only alternative, and has already replaced MB in many situations. However, PH_3 is not always appropriate, particularly because of its requirement for long exposure periods (5 d or longer) (Howe 1974), which makes it unsuitable for rapid disinfestation or quarantine fumigations. Moreover, development of insect resistance to PH_3 (Champ and Dyte 1976, Zettler et al. 1989, Zettler and Cuperus 1990) is a source of concern for its future use. Sulfuryl fluoride is used in some countries to disinfest structures, but has relatively poor ovicidal properties, and a long exposure period (1 d or more) is required for quarantine fumigations (Taylor 1994).

Some other alternatives to MB, such as modified or controlled atmosphere technologies (carbon dioxide and vacuum), inert dusts, cold or heat treatment, and ambient air aeration or refrigerated air aeration may offer some potential for controlling insect pests in stored products, but they do not have short exposure times for disinfestation. MB is apparently the only fumigant available for quarantine treatment of commodities for which rapid disinfestation techniques and a very high degree of insect mortality are essential. The loss of MB could have a significant negative impact on world agriculture, particularly because no alternatives to MB are currently available for rapid disinfestation of commodities. Thus, there is a critical need to develop new fumigants for quarantine purposes.

Propylene oxide (PPO) is a liquid fumigant under normal temperature pressure with a relatively low boiling point (35°C) and a noticeable ether odor (Weast et al. 1986). It is a safe fumigant for use on food as a sterilant because it is quickly converted to nontoxic glycols in the human stomach (Griffith 1999). PPO is approved by the United States Food and Drug Administration as a fumigant to control microbial contamination on certain dry food products such as dry and shelled walnuts, cocoa powder, and spices (Anonymous 1980). A disadvantage of PPO is that it is flammable from 3 to 37% in air, and therefore, to avoid flammability it should be applied under low pressure or in CO_2-enriched atmospheres.

Griffith (1999), in preliminary tests on some stored product pests, indicated that PPO has insecticidal properties under vacuum conditions as a fumigant by killing all stages of the confused flour beetle (*Tribolium castaneum* [Herbst]), the Indianmeal moth (*Plo-*

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dia interpunctella [Hübner]), and the warehouse beetle (*Trogoderma variable* [Everts]). Isikber et al. (2001) and Navarro et al. (2004) 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, because the rate of sorption affects the insecticidal efficacy of the fumigant. Although some preliminary studies on relative effectiveness of PPO at low pressure were done by Griffith (1999), Isikber et al. (2001), and Navarro et al. (2004), only limited information is available on the efficacy of a PPO in combination with low pressure against a wide range of stored product insects. Therefore, the objective of this work was to determine the toxicity of PPO at low pressure against several important stored product insects over a short exposure time. It reports on the results of laboratory experiments using all life stages of the confused flour beetle (*T. castaneum* [Herbst]), the Indianmeal moth (*P. interpunctella* [Hübner]), the Mediterranean flour moth (*Ephestia cautella* [Wlk.]), and the sawtooth grain beetle (*Oryzaephilus surinamensis* [L.]).

Materials and Methods

Test Insects

Tests were carried out on all life stages of the four stored product insects: *T. castaneum*, *P. interpunctella*, *E. cautella*, and *O. surinamensis*. All stages were obtained from laboratory cultures reared at 26 ± 1°C and 70 ± 5% RH (r.h.) on standard cultures (Donahaye 1990). 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 (Navarro and Gonen 1970). Two slides, each containing 100 eggs aged 1–2 d, were exposed to each treatment. Larvae were removed from culture jars and exposed to the treatments 12 d after oviposition. 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 preexposure jars containing wheat flour, and were exposed to treatment 7–10 d after emergence.

Fumigation Chambers

Test chambers consisted of 2.64-liter 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, 6.2 mm ID, were attached to the tubing and sealed with pinch clamps. The desiccators were sealed with silicone vacuum grease.

The Fumigant

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

PPO was introduced as a liquid into the desiccators using a 50 or 250 μl gas-tight syringe. Pressure in each desiccator was measured using a 0–800 mm Hg Celesco-model SE-2000 vacuum gauge (Tedea Ltd., Herzelia, Israel). The 100 mm Hg measure referred to in this work is absolute pressure, with 760 mm Hg considered as atmospheric pressure. Before each test, 50 larvae, pupae, or adults were confined, separately, inside 3-cm-diameter × 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, before 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 insects. For *E. cautella*, dosages were: for adults 1–5 mg/liter, pupae 2.5–15 mg/liter, eggs 1.3–10 mg/liter, and larvae 5–15 mg/liter; for *P. interpunctella*, dosages were: for adults 1–5 mg/liter, pupae 2.5–10 mg/liter, eggs 2.5–20 mg/liter, and larvae 5–15 mg/liter; for *T. castaneum*, dosages were: for adults 5–20 mg/liter, pupae 5–30 mg/liter, eggs 4–30 mg/liter, and larvae 2.5–18 mg/liter; for *O. surinamensis*, dosages were: for adults 2.5–7.5 mg/liter, pupae 2.5–30 mg/liter, eggs 2.5–15 mg/liter, and larvae 1–5 mg/liter. Each test was replicated at least twice. For each test, untreated control insects were exposed to atmospheric conditions. The 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 ± 5% at atmospheric pressure (using saturated magnesium nitrite salt) and 30 ± 1°C, respectively. However, the r.h. decreased to 50 ± 5% at the initial stage when 100 mm Hg was applied, but returned to 65 ± 5% within 30 min. 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 at the end of the 4-h exposure period by withdrawing 15 μl gas sample from the exposure chamber, using a 50 μl gas-tight syringe. The concentration of PPO was measured using a Shimadzu 17A GC (Agntech Ltd., Tel Aviv, Israel) fitted

Table 1. Probit analysis data for PPO at low pressure of 100 mm Hg for all life stages of *O. surinamensis*, *T. castaneum*, *P. interpunctella*, and *E. cautella* resulting from 4-h laboratory fumigations at 30°C

Tested insect species	Life stage	n[a]	Slope[b] ± SE	LD_{50} (fiducial limit)[c] (mg/L)	LD_{99} (fiducial limit)[c] (mg/L)	H[d]
O. surinamensis	Egg	800	3.62 ± 0.54	2.8 (2.09–3.34)	12.2 (9.26–19.29)	0.68
	Larva	368	6.4 ± 2.09	2.1 (1.11–2.40)	4.7 (3.77–11.86)	0.47
	Pupa	294	3.5 ± 0.51	5.7 (4.07–7.11)	26.1 (19.95–40.27)	0.74
	Adult	423	10.45 ± 1.05	4.7 (4.52–4.87)	7.4 (6.72–8.57)	0.98
T. castaneum	Egg	800	6.8 ± 1.26	8.6 (7.34–9.3)	18.9 (16.04–26.44)	0.31
	Larva	222	5.0 ± 0.56	5.1 (4.50–5.72)	14.8 (11.74–20.74)	0.14
	Pupa	254	5.6 ± 0.75	9.8 (7.75–11.76)	25.7 (20.76–34.87)	0.25
	Adult	178	12.5 ± 1.61	6.8 (6.22–7.41)	10.4 (9.15–12.64)	0.01
P. interpunctella	Egg	1,000	6.4 ± 0.66	6.7 (6.03–7.25)	15.3 (13.42–18.48)	0.75
	Larva	236	9.1 ± 1.52	7.7 (7.10–8.38)	13.9 (11.89–18.59)	0.26
	Pupa	276	12.3 ± 2.65	5.7 (5.01–6.25)	8.8 (7.69–11.79)	0.70
	Adult	202	4.7 ± 0.71	1.9 (1.58–2.22)	5.9 (4.41–9.72)	0.89
E. cautella	Egg	800	4.0 ± 0.47	1.6 (1.35–1.79)	6.1 (4.89–8.70)	0.91
	Larva	246	8.3 ± 2.08	6.8 (5.41–7.67)	13.0 (10.79–20.90)	0.94
	Pupa	298	7.8 ± 1.64	7.2 (6.17–8.06)	14.4 (11.94–21.60)	0.17
	Adult	198	7.7 ± 1.77	2.8 (2.17–3.33)	5.7 (4.74–8.77)	0.62

[a] Number treated, excluding controls.
[b] Slopes among life stages of tested insect are unparallel and unequal where noted.
[c] Numbers in brackets give the 95% confidence range.
[d] Heterogeneity factor, χ^2/degrees of freedom (χ^2 is significant, $P < 0.05$).

with an FID (Flame Ionization Detector) and an EC-WAX (Alltech, Deerfield, IL) capillary column (30 m length × 0.25 mm ID × 0.25 μm film thickness) run at 170°C isothermal. During the operation, gas flow rates were 30, 50, and 500 ml/min for helium, hydrogen, and air, respectively. Temperatures were 170°C, 250°C, and 260°C for column oven, injector port, and detector, respectively. Under these conditions, the retention time of PPO was ≈2.65 min. To provide a standard, an empty chamber was injected with five different dosages ranging from 10 to 100 mg/liter, and each response (peak area) was detected by the GC. Thereafter, data were placed into a spreadsheet and analyzed on a computer using SAS statistical software program (SAS Institute 1988) to fit data to the best regression of concentration versus the response (peak area). Data points were then plotted with Jandel Scientific (San Rafael, CA) software program SigmaPlot. This standard curve was applied to each gas sample assay.

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 ± 1°C and 70 ± 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 made 4–5 d after exposure; for larvae they were based on those insects that had failed to pupate 9 d after exposure; pupal mortality was based on those pupae that failed to produce adults 9 d after exposure; and egg hatch was counted 7 d after treatment. Zero dose control and dose-mortality responses were subjected to probit analysis by the POLO-PC computer program (LeOra Software 1987) to determine LD_{50}, LD_{99}, and their respective 95% confidence intervals (CLs). Differences in toxicity were considered significant when 95% CLs did not overlap. The slopes and intercepts of concentration-mortality regressions for each tested insect were compared with the POLO-PC maximum-likelihood procedures (LeOra Software 1987). Required concentrations × time (Ct) products 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 and Discussion

PPO at a low pressure of 100 mm Hg was toxic to all life stages of the four species of stored product insect tested, and each was unique in its inherent susceptibility to the fumigant. Probit mortality regression data for PPO at 100 mm Hg against adult and immature stage of *P. interpunctella*, *E. cautella*, *O. surinamensis*, and *T. castaneum* are shown in Table 1. Hypothesis tests for parallelism and equality (Robertson and Preisler 1992) indicated that all regression lines for the life stages of the four species were neither parallel (g = 0.045, 0.51, 0.132, and 0.092; Heterogeity = 1.91, 1.75, 4.9, and 3.13 for *P. interpunctella*, *E. cautella*, *O. surinamensis*, and *T. castaneum*, respectively) nor equal (g = 0.56, 0.28, 0.33, and 0.12; Heterogeity = 17.05, 16.19, 14.74, and 8.79 for *P. interpunctella*, *E. cautella*, *O. surinamensis*, and *T. castaneum*, respectively). Nonparallel probit lines indicate that all life stages of the four species tested were not responding to PPO at 100 mm Hg in the same manner. Toxicities (LD_{99}) for the life stages of *P. interpunctella* ranged from 5.9 to 15.3 mg/liter, reflecting decreasing susceptibilities in the order of adult, pupa, larva, and egg, respectively; LD_{99} toxicities for the life stages of *E. cautella* ranged from 5.7 to 14.4 mg/liter, reflecting decreasing susceptibilities in the order of adult, egg, larva, and pupa, respectively. However, LD_{99} toxicities for the life stages of *T. castaneum* ranged from 10.4 to 25.7 mg/liter, reflecting decreasing susceptibilities in the order of adult, larva,

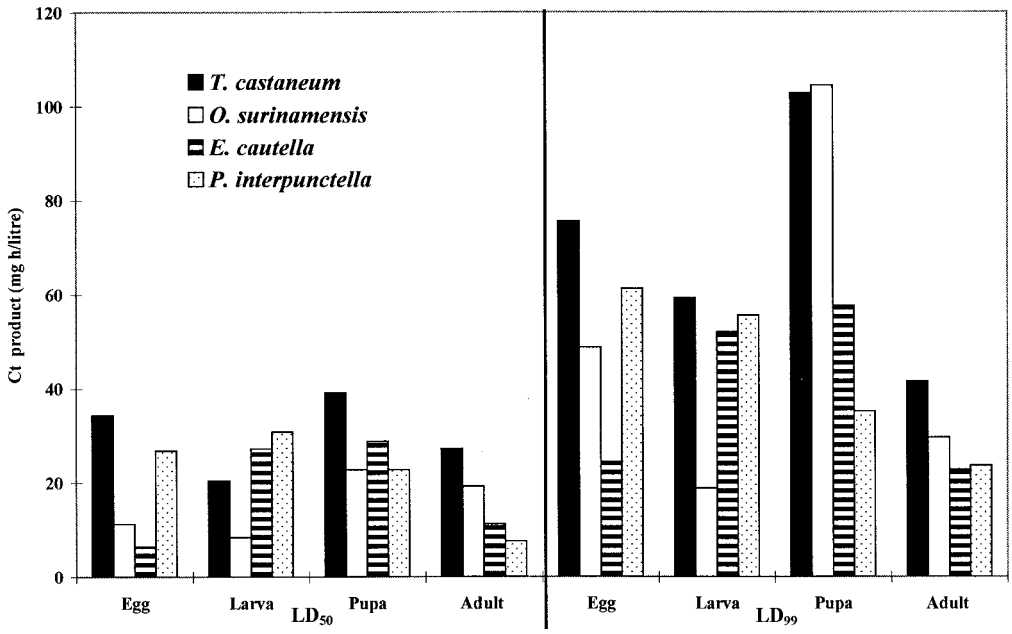

Fig. 1. Comparative Ct products (mg h/liter) required for LD_{50} and LD_{99} values of PPO at low pressure of 100 mm Hg for all life stages of *P. interpunctella*, *E. cautella*, *O. surinamensis*, and *T. castaneum*.

egg, and pupa; LD_{99} toxicities for the life stages of *O. surinamensis* ranged from 4.7 to 26.1 mg/liter, reflecting decreasing susceptibilities in the order of larva, adult, egg, and pupa.

Pupae of *E.* cautella, *O. surinamensis*, and *T. castaneum* were more tolerant than their eggs, larvae, and adults, and had LD_{99} values of 14.4, 26.1, and 25.7 mg/liter, respectively. However, LD_{99} values for the pupae of *E. cautella* and *T. castaneum* were not significantly different from their larvae and eggs, because 99% CLs of *E. cautella* and *T. castaneum* pupae overlapped those of their larvae and eggs. For *P. interpunctella*, eggs were more tolerant than other life stages and had a LD_{99} value of 15.3 mg/liter, although the LD_{99} value for *P. interpunctella* eggs was not significantly different from that of larvae because the 99% CLs for *P. interpunctella* eggs overlapped those of larvae. In general, the eggs and pupae of the tested species were more tolerant than the adults and larvae, the exception being *P. interpunctella.* Similarly, records show that the egg and pupal stages of stored product insects are generally more tolerant than larvae and adults to carbon dioxide (AliNiazee 1971) and to fumigants MB (Hole 1981), phosphine (Howe 1974), and carbonyl sulfide (Plarre and Reichmuth 1997).

T. castaneum and *O. surinamensis* required much higher dosages of PPO than those of *P. interpunctella* and *E. cautella* to obtain 99% mortality of their life stages (Table 1). The 99% mortality of all life stages of *O. surinamensis* and *T. castaneum* was achieved at a concentration of 26.1 and 25.7 mg/liter, respectively, whereas that of all life stages of *P. interpunctella* and *E. cautella* was obtained at 15.3 and 14.4 mg/liter, respectively. The order of tolerance of the pupal stage at the LD_{99}, which is generally the most tolerant stage to fumigants, was: *O. surinamensis* > *T. castaneum* > *E. cautella* > *P. interpunctella.*

Calculations of Ct products reveal also that there was a high variation in Ct products of the different life stages at the LD_{50} and LD_{99} levels (Fig. 1). Ct products of 22.8, 39.2, 28.8, and 22.8 mg h/liter were required to obtain 50% kill of pupae of *O. surinamensis*, *T. castaneum*, *E. cautella*, and *P. interpunctella*, respectively (Fig. 1), while Ct products of 104.4, 102.8, 57.6, and 35.2 mg h/liter were required for 99% kill for adults of *O. surinamensis*, *T. castaneum*, *E. cautella*, and *P. interpunctella*, respectively. These findings may be compared with several studies on the two most commonly used fumigants, MB and PH_3, for control of *T. castaneum.* MB and PH_3 require Ct products of 53.3 and 31.5 mg h/liter, respectively, to obtain 50% of kill of the pupae (Bang and Telford 1966). Other fumigants such as ethylene dichloride and carbon tetrachloride produced 50% mortality of the pupae of *T. castaneum* with Ct products of 421.4 and 1091.2 mg h/liter, respectively (Bang and Telford 1966, Busvine 1938). Tests of carbonyl sulfide on adults of *O. surinamensis* produced 50% mortality at a Ct product of 143.5 mg h/liter (Zettler et al. 1997), while PPO at low pressure required only a Ct product of 19.2 mg h/liter to obtain 50% kill of adults in this study (Fig. 1). It appears also that PPO combined with low pressure is less toxic to pupae of *T. castaneum* than PH_3, but is more toxic than MB, ethylene dichloride, and carbon tetrachloride.

The results obtained in this study indicate that PPO at low pressure can provide 99% mortality of all life stages of the insect species tested at a Ct product of 104.4 mg h/liter, indicating that it is even more toxic as a space fumigant than MB. Because these results

show that PPO at low pressure is highly toxic to insects within a short exposure time, it becomes apparent that the combination of PPO with low pressure can be a potential alternative to MB for quarantine treatment of commodities in which rapid disinfestation and a very high degree of insect mortality are essential.

In addition to high and rapid toxicity to insects, a fumigant should have a reasonable sorption by the commodities, rapid penetration into bulk commodities, and no adverse impact on commodity quality. Although these toxicity tests indicate that the combination PPO with low pressure is a rapidly acting treatment for disinfestation of commodities, it is clear that further studies are needed to obtain data on phytotoxicity of PPO on seeds and fresh commodities, on its absorption by different commodities, and on its power of penetration into bulk commodities. Moreover, because most quarantine treatment schedules include temperature scales much lower than that used in this study (30°C), further toxicity tests of PPO with low pressure at other temperatures need to be done to determine its potential as a fumigant for quarantine treatment under a wide range of conditions.

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