Plant Biosecurity

Cooperative Research Centre

A report prepared for BOC Limited:

Comparison of ethanedinitrile (EDN) with methyl bromide (MB) as a biosecurity fumigant for timber and log

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1. Introduction

Fumigation has a long history in the disinfestation of timber and wooden structures.

In quarantine settings, one of the beneficial characteristics of fumigants is that they reach points on wood bundles and, to varying degrees, inside wood that other pesticides do not easily reach.

Methyl bromide (MB) is widely used for quarantine treatment of timbers, wooden packaging and logs (Ren, 1996; Ren et al., 1997; Ren et al., 2006).

Sulfuryl fluoride (SF) also has a long history for the control wood-destroying termites (targeting adults and queens) in structures (Su and Scheffrahn, 1986) but is not as useful in biosecurity / quarantine settings as it has lower toxicity to the egg stage of insects.

Industry and government are progressively losing access to MB as it is withdrawn as an ozone depleting substance under the Montreal Protocol (UNEP, 2006). Therefore there is an urgent requirement for the development of a replacement fumigant which can effectively control insects in timber.

One such candidate is ethanedinitrile (EDN) which is registered in Australia by BOC Limited and which has been shown to have potential as a quarantine treatment for timber (Viljoen and Ren, 2001; Wright et al., 2002; Ren et al., 2006; Ren and Lee, 2008).

To pursue the use of EDN as a fumigant in biosecurity settings Mr Wayne Riley and Mr David Rees from Department of Agriculture, Fisheries and Forestry, Australia met with Prof Yong Lin Ren, Murdoch University to generate a research protocol, inclusive of supporting bioassays, for the purposes of comparing the relative efficacies of EDN and MB.

Building from this BOC provided financial support for Prof YL Ren, under the auspices of the Plant Biosecurity CRC, to test the two fumigants (EDN and MB) as per the agreed protocol.

This report details:

- The findings of the BOC-supported experiments as undertaken per the agreed protocols; and
- The resulting comparison of EDN and MB as quarantine fumigants for timber and log(s).

2. Executive summary

Murdoch University's Postharvest Biosecurity and Food Safety Laboratory were engaged by BOC Limited, under the auspices of the Plant Biosecurity CRC, to compare the efficacy of ethanedinitrile (EDN) and Methyl Bromide (MB) in quarantine settings as per a protocol developed in consultation with Mr Wayne Riley and Mr David Rees from Department of Agriculture, Fisheries and Forestry.

The research was conducted to evaluate the toxicity of EDN (a fumigant patented by CSIRO Australia and commercialised by BOC Limited) and MB.

The research generated new data on the penetration of EDN and MB through timber blocks, concentration × time (Ct) in both a fumigation chamber and in timber block cores, and sorption rate of the two fumigants on timber under laboratory controlled experimental conditions.

The key findings from the research were:

- EDN is more toxic than MB to all life stages of the five test insect species under laboratory controlled experimental conditions.
- EDN can penetrate to a depth of 15 cm in soft timber after 24 hours fumigation.
- The amount of EDN present in soft timber diminishes with depth.
- EDN can penetrate to a depth of 15 cm in hard timber after 24 hours fumigation. However this result is dependent on the thickness of the timber; at a timber thickness of 20x20x30 cm EDN penetration is limited to a depth of 10cm.
- MB does not penetrate as well as EDN. MB will penetrate to a depth of 15 cm in soft timber but at a lower concentration compared to EDN; MB does not penetrate beyond a depth of 5 cm in most situations in hard timbers.
- On this basis it is recommended that MB be used only as a shallow surface treatment (at depths not exceeding 5 cm from the timber surface).
- EDN provides better control of *T. variabile* larvae and mixed stages of *T. castaneum* than MB at low temperatures.

In conclusion, based on an agreed protocol, EDN outperformed MB as a quarantine fumigant for the control of all five tested insect species in timber under laboratory controlled experimental conditions.

3. Aims

This project was delivered in two stages, phase 1 and phase 2 / 3.

Phase 1 of the project reported comparative results of mortality and concentration ×time p (Ct) products of EDN and MB by evaluating toxicity of these fumigants to all life stages of five mixed-age cultures of timber insects.

The aims were to:
Conduct laboratory bioassays to determine toxicity of ethanedinitrile (EDN) to all life stages of mixed age cultures of *Lasioderma serricorne*, *Sitophilus oryzae* (L.), *Ryzopertha dominica, Tribolium castaneum* and *Trogoderma variabile* Ballion

Phase 2 and 3 of the project generated comparative results for the penetration of EDN and MB through timber blocks, concentration x time p (Ct) products in the fumigation chamber and in the timber block core under laboratory controlled experimental conditions.

The aims were to:

1. Evaluate (in the laboratory) the toxicity of EDN and MB against diapausing larvae of warehouse beetles (*Trogoderma variabile*) at 5^{o}C and 15^{o}C
2. Determine the penetration of EDN into timber blocks and logs
3. Conduct laboratory scale trials to evaluate EDN fumigation of timber logs and efficacy in comparison with MB

4. Materials and Methods

4.1 Phase 1

4.1.1 Insects

The standard of quarantine security for many importing countries is probit-9, or 99.996832% mortality of a pest population. In practical terms, probit-9 means that only 32 individuals can survive out of 1 million treated insects (a number that is impossible to achieve in both laboratory and field bioassays). Some countries require a specific number of test insects to be treated during confirmatory studies and 5,000-10,000 test insects with no survivors are commonly used. This number of test insects is difficult, if not impossible, to apply for both laboratory and field bioassays with timber insects. In order to obtain a fairly high population of insects for testing, this study chose mixed-age cultures (including; adults, pupae, larvae and eggs) of the following test insects:

- Cigarette beetle, *Lasioderma serricorne*
- Rice weevil, *Sitophilus oryzae* (L.)
- Warehouse beetle*, Trogoderma variabile* Ballion
- Lesser grain borer, *Rhyzopertha dominica* (F.)
- Rust red flour beetle, *Tribolium castaneum* (Herbst)

L. serricorne, *T. castaneum* and *T. variabile* were chosen because their adults or pupae or larvae or eggs have a high tolerance to MB (Table 1) and they provided broad coverage of the beetle families related to wood borers. *R. dominica* and *S. oryzae* are common pests of stored grain. In eastern Australia, strains of both species have developed resistance to phosphine.

The tested insect species of *L. serricorne, S. oryzae, T. castaneum, T. variabile* and *R. dominica* were the phosphine and MB susceptible strains MULS1, MUSO1, MUTC1, MUTV and MURD2 respectively, held at the Post-harvest Plant Biosecurity Laboratory, Murdoch University, Australia. The techniques of insect culturing and handling generally follow those described by Winks (1982) for *T. castaneum*. All five mixed-age cultures of the test species were established by adding adults (400-500) to media (1 kg) at 25°C and 65% relative humidity (RH). *S. oryzae* was reared on wheat. *T. castaneum and L. serricorne were* reared on medium comprising 1 part yeast and 12 parts wholemeal flour milled from Australian soft wheat (Rosella). *R. dominica* was reared on medium containing 40 parts wheat and 1 part the wholemeal flour. *T. variabile* was reared on crushed canola. Prior to grinding or use for rearing, the wheat, wholemeal flour and crushed canola were conditioned to 12.5% m.c (wheat and wholemeal flour) and 6.0% m.c (crushed canola) and disinfested by freezing at -20°C for >2 days. The adults were left on the media for 4-5 weeks. After this period and based on our knowledge of development rates there were representative numbers from each life cycle stage - egg, larva, pupa, and adult.

Table 1. Susceptibility of different stages of five insect species to MB

Insect stages	Tolerance of insect species to methyl bromide Most tolerant → Least tolerant				
Adults	*T. castaneum*	*T. variabile*	*S. oryzae*	*R. dominica*	*L. serricorne*
Pupae	*T. castaneum*	*T. variabile*	*L. serricorne*	*S. oryzae*	*R. dominica*
Larvae	*L. serricorne*	*T. variabile*	*T. castaneum*	*S. oryzae*	*R. dominica*
Eggs	*L. serricorne*	*T. castaneum*	*T. variabile*	*S. oryzae*	*R. dominica*

4.1.2 Fumigants

EDN (99.0% C_2N_2 and 1.0% air and CO_2) and MB (98.5% methyl bromide and 1.5% air) were sourced from BOC Gases Australia.

The dosages and required volumes for the fumigant concentrations were calculated from Eq. 1 calibrated to the laboratory temperature and pressure.

$$V_f = \left(1 + \frac{T}{273}\right)\left(\frac{1.7 \times 10^6 \times C \times V}{P \times M \times N}\right) \qquad \text{Eq. 1}$$

Where: ***V*** is volume of fumigation container (L)

P is pressure (mm Hg)

T is temperature (°C)

C is the intended concentration of fumigant (mg L^{-1})

$\mathbf{V_f}$ is dosage volume of fumigant (mL)

M is molecule weight of fumigant, and

N is purity of gas (%)

4.1.3 Measurement of temperature and relative humidity

During the fumigation, temperature and RH were automatically recorded with a HOBO® data logger unit, (Model number H08-004-02, Onset Computer Corporation, MA 02532, USA, www.onsetcomp.com) in each of the fumigation chambers. The recorded data was read with the software BoxCar® Version 3.6+ for Windows (Onset Computer Corporation, MA 02532, USA, www.onsetcomp.com). The HOBO®s was checked for calibration in the laboratory against each other and a standardised mercury glass thermometer, as well as a range of glycerol/water solutions for relative humidity.

4.1.4 Measurement of EDN and MB

During fumigation, concentrations of EDN and MB were monitored at time intervals (from 10 min after application until opening the bottles) over the exposure period (6 and 24 hours).

The EDN and MB concentrations were determined on a Varian 3600Cx Gas Chromatograph (Varian Associates, Inc., USA) equipped with a flame ionization detector (GC-FID) (Figure 1) after isothermal separation on a megabore capillary column (DB FFAP, J&W 125-3232), 30 m×0.53 mm i.d. with the oven temperature set at 100°C.

Figure 1. The concentration of EDN and MB in fumigation containers was analysed with Gas Chromatograph (GC) equipped with Flame Ionisation Detector (FIP)

Gas standards were prepared by an injection of a known volume of concentrated gas EDN and MB into 1 litre Erlenmeyer flasks (Bibby Sterilin, Staffordshire, Cat. No. FE 1 L/3) equipped with a cone/screw-thread adapter (Quickfit, STS; Bibby Sterilin) each containing five glass beads (2-3 mm o.d.). The volume of each Erlenmeyer flask and inlet system was measured from the weight of water required to fill the container. The volume of fumigant used was calculated from Eq. 1. After mixing, the diluted gases were injected into the GC to obtain a calibration based on peak areas. A fumigant sample volume of 60 µL was injected

manually into the GC, and the concentrations were calculated on the basis of peak areas against the calibrated gas standards which were also read periodically during the exposure period.

4.1.5 Determination of concentration × time products (*Ct*)

The concentration × time (*Ct*) products were calculated from the arithmetic average of EDN and MB concentration readings during the 6 and 24 hour exposure period. The *Ct* products were calculated from Eq. 2.

$$Ct = \sum (C_i + C_{i+1})(t_{i+1} - t_i)/2 \qquad \text{Eq. 2.}$$

Where: **C** is fumigant (EDN and MB) concentration (mg liter^{-1})

t is time of exposure (hours)

i is the order of measurement

Ct is concentration × time products (mg h liter^{-1})

4.1.6 Measurement of carbon dioxide

Carbon dioxide and oxygen were monitored before the injection of fumigant and at 6 and 24 hours after the injection of fumigant with Witt OXYBABY® 6.0 (WIT-GasetechnikGmbH & Co KG T, Germany). Accuracy 0.1-100% O_2/0.01-100% CO_2.

4.1.7 Fumigation procedure

The fumigation was conducted in 250 mL Erlenmeyer flasks equipped with ground-glass joint and septum sampling system (Bibby Sterilin, Staffordshire, UK; Cat. No. FE 250/3) containing 50 g mixed-age cultures (containing more than 100 countable adult insects and uncountable immature stages of eggs, larvae and pupae) with 20% of loading rate at 25ºC and 65% RH. An indication of the number of eggs of each species present in the media can be obtained from the numbers of insects that emerged after 35 days from control treatments (Tables 3 and 4). After removal of the same volume of air as the injected fumigant, the fumigant was injected into the bottle. The dosage (calculated by Eq. 1) was injected into the bottles using a gas-tight syringe. The control was maintained in a sealed bottle without fumigant until completion of exposure. Each fumigant treatment was at 5-7 levels of fumigant and in 5 replicates (n=5) and 3 controls. At the end of the fumigation period of 24 hours, the treated and control bottles were opened for 1 hour of aeration. The insects were transferred to new bottles (50 mL), 25% full of fresh media, and then incubated at 25°C and 70-75% RH.

4.2 Phase 2 and 3

4.2.1 Insects

The tested insect species of *T. castaneum*, *S. oryzae* and *T. variabile* used were a phosphine / MB susceptible strain known as MUTC1, MUSO1 and MUTV which were held at the Post-harvest Plant Biosecurity Laboratory, Murdoch University. *T. variabile, S. oryzae* and *T. castaneum* were reared as described under section 3.1.1.

4.2.2 Prepare timber blocks

Three types of soft timber, pinewood (not Kiln dried Douglas, Radiata and Merbau) and Eucalyptus species (Jarrah) were used as the source of the timber blocks for testing.

Timber blocks (10 cm × 10 cm × 30 cm and 20 cm × 20 cm × 30 cm) were cut with one axis on the longer side parallel to the grain and cut at least 5 cm from the end of the piece of lumber (Figure 2). The blocks had no visible cracks at the time of preparation. Before starting the fumigation testing, the timber blocks were conditioned for 3 weeks at 25±2°C, 55% and 85% relative humidity (r.h.) to obtain dry timber and high moisture content ("green") sawn wood related samples. Table 2 displays the moisture content and timber block density that was determined with GE Protimber-Surveymaster (range of measurement 1-35% with ±0.3% accuracy) – deployed by piercing two pins attach at the front of the instrument into the wood.

Table 2. The moisture content and density of the soft and hard timber blocks

	Dry timber		High moisture content sawn wood	
	Moisture content (%)	Density (g cm^{-3})	Moisture content (%)	Density (g cm^{-3})
Douglas	12.7	0.55	19.7	0.65
Radiata	13.5	0.56	19.1	0.64
Merbau	13.2	0.57	19.9	0.67
Jarrah	9.9	0.95	14.3	1.2

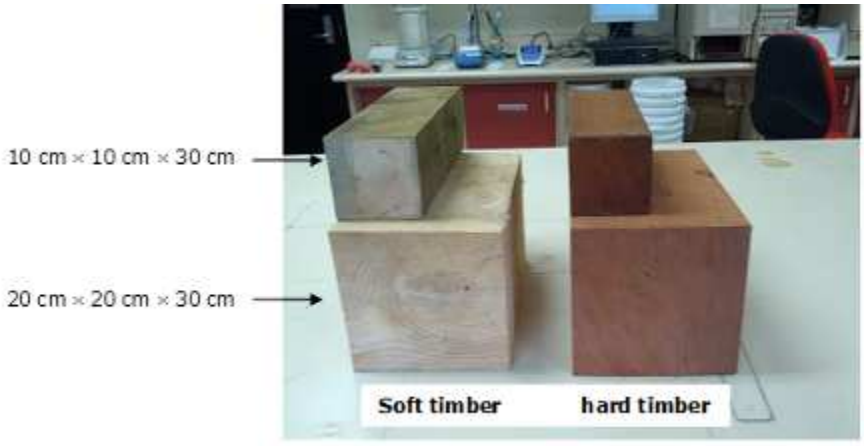

Figure 2. Soft and hard timber blocks with different sizes and sampling ports

The major route of penetration of MB into softwood timber blocks is with the grain of the timber block rather than across the grain (Brenton, 1990; Cross, 1991; Ren, 1996; Ren et al., 1997). Therefore, for testing the movement of fumigants (EDN and MB) in timber, each timber block had one set of sampling holes fitted with gas sampling ports (Figures 3-6). A set of gas monitoring holes were placed at 5, 10, 15, 20 and 25 cm from one end of the timber block along the middle of the timber block. For testing of penetration across the grain both ends of the timber block were sealed with silicone.

In order to take gas samples during the fumigation process out of the timber blocks, the top plate of the fumigation chamber was furnished with 10 gas sample probe fittings, sealed by screwed-on septa as commonly used in gas chromatographs. The holes were of 1.5 mm i.d. and were lined with gas sampling probes (5-10 cm long × 3.0 mm o.d.) to facilitate sampling of gas directly from the centre of the block and to ensure at least 4-5 mm between the end of the probe and bottom of hole. This made the sampling volume more than 200 µL. The gas sample system (gas sample probe and sealing) were fitted into the timber block before being placed into the top plate of the fumigation chamber. The sample port septum and cap were fitted after hooking the timber block to the top plate (Figures 5-6).

Figure 3. Timber blocks (10×10×30 cm) are in the position for testing and sample ports are 5, 10, 15, 20 and 25 cm from one end of the block and the other end is sealed with silicone.

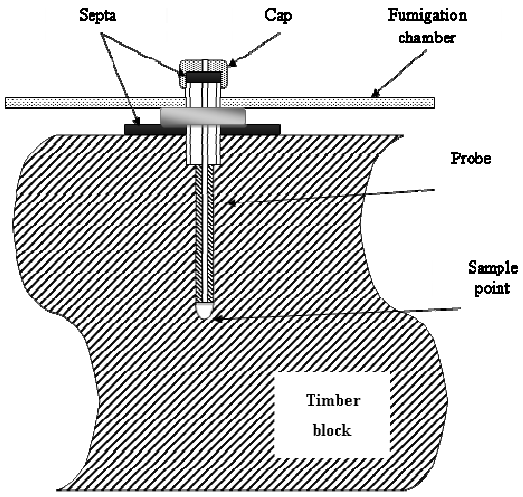

Figure 4. Schematic representation of sealing, gas sample probe fitting and sample port.

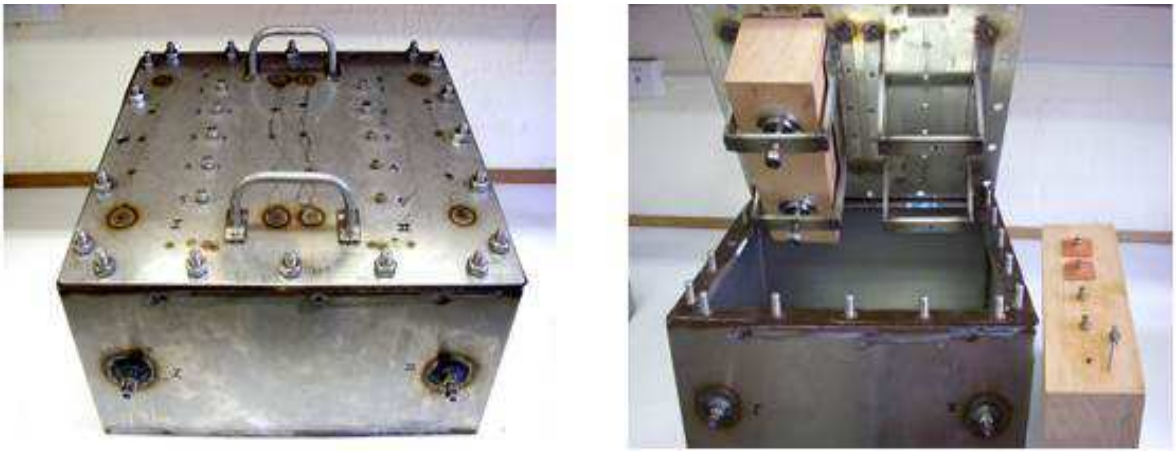

Figure 5. Photos of a sealed fumigation chamber (20×40×18.75 cm) fitting and sample port (above) and opened chamber shows the organisation inside the chamber; timber blocks (10×10×30 cm) are in the position for testing and sample ports and probes (right).

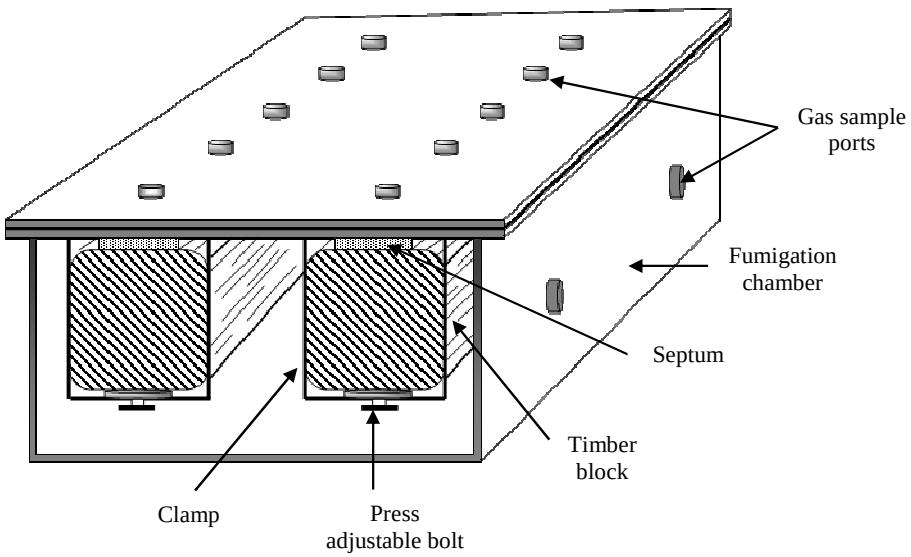

Figure 6: Schematic representation of a sealed fumigation chamber and the organisation inside the chamber; timber blocks are in the position for testing and sample ports are 5, 10, 15, 10 and 5 cm from both end of the block and 5, 10, 15, 20 and 25 cm from one end (other end sealed with silicone).

To evaluate fumigant efficacy within timber, a number of holes (Ø3 cm) and 5-10cm depth cross grain were prepared. Insect samples were placed in the holes. The hole was sealed with a tight plug made from the same timber type (Figure 7).

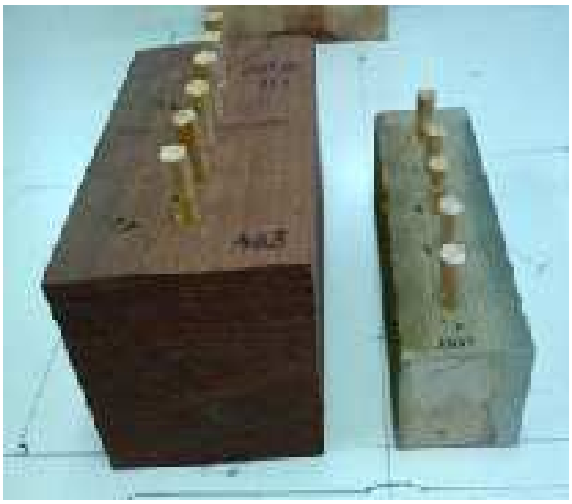

Figure 7. The timber block for evaluation of fumigant efficacy to kill insect within timber

4.2.3 Fumigation chamber

Two types of fumigation chambers were prepared for testing two size timber blocks (cross-sectional sizes of 10x10x30cm and 20x20x30cm). The size of the fumigation chambers is shown in Table 3.

Table 3. The size of the timber blocks and fumigation chambers

cross-sectional sizes of timber block (H×W×L)	fumigation chamber (H×W×L)	volume of the chamber	Load timber block	loading ratio
10x10x30 cm	20×40×18.75 cm	15 L	1	20%
10x10x30 cm	20×40×18.75 cm	15 L	2	40%
20x20x30 cm	30×50×40 cm	60 L	1	20%
20x20x30 cm	30×50×40 cm	60 L	2	40%

The fumigation chamber was made from stainless steel and fitted with 2 gas sampling ports (Figure 5). The net volume of the chamber was 15 and 60 L. The top plate of the fumigation chamber was designed to accommodate 1-2 timber blocks which are secured on the plate with two clamps for each block (Figures 4-5). One timber block (1×3 L = 3 L and 1×12 L = 12) was placed in 15 and 30 L chamber to achieve a loading ratio of 20%. Two timber blocks (2×3 L = 6 L and 2×12 L = 24) were placed in 15 and 30 L chamber to achieve a loading ratio of 40%.

The design loading ratio of 20% is based on a fully loaded container of pallets (the pallet timber taking <20% of the container volume). In the case of commercial container fumigation, the timber pallet often only occupies less than 5% of the container volume. A 20% loading ratio is therefore designed to reflect the treatment of pallets only (i.e. a container fully loaded with pallets). The 40% loading ratio for 10x10x30 cm and 20x20x30 cm timber block designed is based on a fully loaded with saw timber (Figure 5).

For fumigation of timber blocks containing insects, steel fumigation drums (47.8 cmx40 cm id) were used with a net volume of the chamber of 60 litres (Figures 8). For recirculation, each drum was equipped with Swagelok compression fittings for attachment to a diaphragm pump just above

the bottom rim and just below the top rim of the drum. The removable lids were fitted with a centrally located septum fitting for introduction of gas during fumigation. The fumigation chamber was fitted with 2-6 gas sampling ports which are located on the side of the fumigation chamber (Figure 8). Each lid is fitted with gas-tight seals which are pressure-tested before use. Pressure halving time of these fumigation chambers was greater than 3 minutes.

Figure 8. The fumigation chamber (47.8x40 cm id = 61 L) equipped with gas injection and sampling systems for laboratory scale trials.

4.2.4 Fumigants

EDN (99.0% C_2N_2; 1.0% air and CO_2) and MB (98.5% MB; 1.5% air) were sourced from BOC.

The selected dosage of MB of 48 g/m^3 was based on the AQIS Methyl Bromide Fumigation Standard (2008) and the EDN dosage of 48 g/m^3 was chosen partly for comparison with MB and partly because both fumigants at these doses control most timber pests (Barak et al., 2002; Ren et al., 2006).

The volume was calculated from that of the total enclosure (commercial volume), not that occupied by the timber. Before dosing, to avoid changes in pressure, a volume of air was removed from the fumigation chamber equivalent to the dosage volume. The dosage (calculated by Eq. 1) of EDN and MB was injected into the fumigation chamber using a gas-tight syringe. Each fumigant treatment is in duplicate (n=2).

The period of fumigation was 24 hour for penetration study and 6 and 24 hours for bioassay. After fumigation, the top of the chamber was opened and aired for two days. Penetration experiments were conducted at 25°C and bioassay at both 15 and 25°C.

The dosages and required volumes for the fumigant concentrations were calculated from Eq. 1 calibrated to the laboratory temperature and pressure.

$$V_f=\left(1+\frac{T}{273}\right)\left(\frac{1.7\times10^6\times C\times V}{P\times M\times N}\right) \qquad \text{Eq. 1}$$

Where: ***V*** is volume of fumigation container (L)

P is pressure (mm Hg)

T is temperature (°C)

C is the intended concentration of fumigant (mg L^{-1})

$\mathbf{V_f}$ is dosage volume of fumigant (mL)

M is molecule weight of fumigant, and

N is purity of gas (%)

4.2.5 Measurement of fumigant in the fumigation chamber and in timber block cores

During fumigation, EDN and MB concentrations were determined on a Varian 3600Cx Gas Chromatograph (Varian Associates, Inc., USA) equipped with a flame ionization detector (GC-FID) (Figure 9) after isothermal separation on a megabore capillary column (DB FFAP, J&W 125-3232), 30 m×0.53 mm i.d. with the oven temperature set at 100°C.

Figure 9. The concentration of EDN and MB in fumigation containers was analysed with Gas Chromatograph (GC) equipped with Flame Ionisation Detector (FID)

For assessment of the penetration into timber blocks and proportion of flow with and across the grain, the ratio of in-timber block to fumigant concentration in the test chamber space provided a measure of penetration. The volume of gas samples taken from each gas sample port and inject into the GC using a 100-µL syringe (Alltech Associates, Sydney, Australia, Cat. No. 005250SGE) was 60 µL. Similar volumes were used with the standard injections. The concentration of the standard was as close as possible to that of the test sample injection.

The concentrations of EDN and MB were monitored at timed intervals (from 10 min after application until opening the bottles) over the exposure period (24 hours), and was used to calculate the product *Ct* = concentration × time (Equation 2).

Gas standards were prepared by an injection of a known volume of concentrated gas EDN and MB into 1 litre Erlenmeyer flasks (Bibby Sterilin, Staffordshire, Cat. No. FE 1 L/3) equipped with a cone/screw-thread adapter (Quickfit, STS; Bibby Sterilin) each containing five glass beads (2-3 mm o.d.). The volume of each Erlenmeyer flask and inlet system was measured from the weight of water required to fill the container. The volume of fumigant used was calculated from Eq. 1. After mixing, the diluted gases were injected into the GC to obtain a calibration based on peak areas. A fumigant sample volume of 60 µL was injected manually into the GC, and the concentrations were calculated on the basis of peak areas against the calibrated gas standards which were also read periodically during the exposure period.

4.2.6 Determination of concentration × time products (Ct)

The concentration × time (*Ct*) products were calculated from the arithmetic average of EDN and MB concentration readings during the 6 and 24 hour exposure period. The *Ct* products were calculated from Equation 2.

$$Ct = \sum (C_i + C_{i+1})\ (t_{i+1} - t_i)/2 \qquad \text{Equation 2.}$$

Where: **C** is fumigant (EDN and MB) concentration (mg liter^{-1})

t is time of exposure (hours)

i is the order of measurement

Ct is concentration × time products (mg h liter^{-1})

4.2.7 Fumigation procedure

Before dosing, the gas-tightness of the fumigation chamber was checked by pressurising and monitoring the gas pressure using a digital manometer (Model EMA 84, Halstrup-Walcher GmbH, Kirchzarten, Germany).

Air (500 mL) was injected into the chamber and drum with 1 L syringe (Alltech Associates, Sydney, Australia, Cat. No. 009770SGE), there is no change in pressure over this period. A volume of air was removed from the fumigation chamber equivalent to the dosage volume to avoid changes in pressure. The dosage (calculated using Eq. 1) was injected into the fumigation chamber, drum and flask using a gas-tight syringe.

For evaluation of fumigant efficacy at 5 and 15ºC, the fumigation was conducted in 250 mL Erlenmeyer flasks equipped with ground-glass joint and septum sampling system (Bibby Sterilin, Staffordshire, UK; Cat. No. FE 250/3). One hundred (100) *T. variabile* larvae were introduced at temperatures of 5 and 15ºC; conditions were at 65% RH. EDN and MB concentrations were monitored to calculate the Ct product. The control was maintained in a sealed bottle without fumigant until completion of exposure.

For evaluation of fumigant efficacy to kill insect within timber, bioassays were conducted by placing 20 *T. variabile* larvae and *S. oryzae* pupa in each hole (Ø3 cm). Based on the bioassay data from phase 1 findings, *T. variabile* larvae and *S. oryzae* pupa were most tolerant to EDN (Table 6) and were used for these tests. The hole was sealed with a tight plug. The fumigation was conducted at 15 and 25±2°C for 24 hour's exposure. The control was treated at the same conditions, but without adding fumigant. After fumigation, the top of the drum was opened and aired for one day and then bioassay samples were collected. The control was maintained in a sealed drum without fumigant until completion of the exposure period.

Each fumigant treatment was at 5-9 levels of fumigant and in 5 replicates (n=5) and 3 controls. At the end of the fumigation period of 6 and 24 hours, the treated and control bottles were opened for one day of aeration. The insects were transferred to new bottles (50 mL), 25% full of fresh media, and then incubated at 25°C and 70-75% RH.

4.2.8 Mortality assessments

Mortality was assessed by counting dead and live *T. variabile* larvae insects after a 24 hour recovery time. The larvae insects were counted and removed, for estimation of endpoint mortality, treatment and control mortalities were continually assessed during 2 and 3 days post treatment. For mixed stages of *T. castaneum,* bioassay samples were retrieved at the end of the fumigation period, the adult insects were counted and removed and the remaining mixed-age cultures incubated at 25°C and 70% r.h. Subsequent emerging adult insects were counted weekly for a period of 6 weeks, with live and dead adults removed at each count. The treatments were compared with the control held at the same exposure temperature and RH.

4.2.9 Statistical analysis

The variations (Standard Deviation) of EDN, MB and CO_2 concentrations in each fumigation chamber and the duplicate injections and *Ct* products for different insects and fumigants in comparison with average readings analysed using Microsoft Excel 2007.

The toxicity of EDN and MB to *T. castaneum*, *R. dominica, S. oryzae* and *L. serricorne* adults (containing unknown number of internal stages) and *T. variabile* larvae insects were estimated from the level of mortality in 3,000 – 4,000 larvae insects exposed to different concentrations. The mortality rate was adjusted for the control insects as mortality is typically less than 1% over a 6 and 24 hour period.

5. Results & Discussion

5.1 Phase 1

5.1.1 Temperature and RH

The variation of temperature and RH during conditions were 25.0±0.5°C and 55.0±2.0% and 25.0±1.0°C during the 6 and 24 hour exposure periods.

5.1.2 Concentrations of fumigants and carbon dioxide during exposure

The media such as wheat, wholemeal flour and crushed canola absorbed EDN and MB during 6 and 24 hours exposure (Figures 10, 11, 12 and 13 - Appendices). The variation of measured concentrations of EDN and MB in the fumigation chambers were constant for each level of dosage. The concentration of EDN and MB varied 4.6±0.7 and 5.1±0.3%, respectively. The concentration of EDN and MB gas standards in glass bottles had no change during 6 and 24 hours exposure. Therefore, the mean value of the concentration can be used to calculate the Ct products with the standard error in fumigation concentration less than 5% of the mean value in all cases.

In both 6 and 24 hours fumigated chambers, the range of CO_2 concentrations slightly increased in comparison with the CO_2 concentration of 0.03% in ambient air'. During these experiments the CO_2 concentrations were always less than 0.8% ensuring the EDN and MB toxicity results were not compromised by CO_2.

5.1.3 Observed toxicity of EDN and MB

Comparison of toxicity between EDN and MB to five species of insects for 6 and 24 hours of exposure at 25°C and 65% RH is shown in Tables 4, 5 and 6. Complete mortality was obtained in all mixed-age cultures of *T. castaneum, R. dominica, S. oryzae, T. variabile* and *L. serricorne* (Table 5). Both MB and EDN were efficacious for all the life stages of five tested insect species.

However, the observed concentration x time (Ct) products of EDN to completely kill all life stages of the five insect species were substantially lower than that for MB for both 6 and 24 hours fumigation. That is, toxicity of EDN was substantially greater than that of MB. In particular, EDN was highly toxic to all the life stages of *T. castaneum* (4-5 times >MB), *R. dominica* and *T. variabile* (about 2 times > MB), adult stages of *S. oryzae* and all immature stages of *L. serricorne* (about 2 times > MB). The emergence of immature stages in the control was very high in comparison with the number of initially added adults. The Ct products between 6 and 24 hours exposure were relatively consistent which indicates that there is a linear relationship for EDN concentration (C) and exposure time (t).

Table 4. Insects emerging from media containing adults, pupae, larvae and eggs following no treatment (control), and exposure to EDN and MB for 6 hours.

Species	Treatment	Before fumigation	After fumigation		Further live insects emerging after holding for (days)				
		Live	Live	Dead	7	14	21	28	35
Tribolium castaneum	Control	2415	2410	0	576	348	672	425	6064
	EDN	3522	0	3522	0	0	0	0	0
	MB	3474	0	3474	0	0	0	0	0
Rhyzopertha dominica	Control	3286	3286	0	615	279	581	279	5418
	EDN	3847	0	3847	0	0	0	0	0
	MB	4015	1	4014	0	0	0	0	0
Sitophilus oryzae	Control	2018	2018	0	492	411	606	371	5104
	EDN	4102	0	4102	0	0	0	0	0
	MB	3916	0	3916	0	0	0	0	0
Lasioderma serricorne	Control	2176	2176	0	251	487	269	195	3126
	EDN	3573	0	3573	0	0	0	0	0
	MB	3721	0	3721	0	0	0	0	0
Trogoderma variabile	Control	2485	2483	2	174	319	451	372	3026
	EDN	3816	0	3816	0	0	0	0	0
	MB	3923	0	3923	0	0	0	0	0

Table 5. Insects emerging from media containing adults, pupae, larvae and eggs following no treatment (control), and exposure to EDN and MB for 24 hours.

Species	Treatment	Before fumigation	After fumigation		Further live insects emerging after holding for (days)				
		Live	Live	Dead	7	14	21	28	35
Tribolium castaneum	Control	3591	3591	0	309	356	239	521	4158
	EDN	4209	0	4209	0	0	0	0	0
	MB	4114	0	4114	0	0	0	0	0
Rhyzopertha dominica	Control	3708	3706	2	314	273	330	362	5025
	EDN	4150	0	4150	0	0	0	0	0
	MB	4283	1	4282	0	0	0	0	0
Sitophilus oryzae	Control	4011	4011	0	372	291	406	317	4519
	EDN	4112	1	4111	0	0	0	0	0
	MB	3961	0	3961	0	0	0	0	0
Lasioderma serricorne	Control	2516	2516	0	194	286	331	528	3207
	EDN	3024	2	3022	0	0	0	0	0
	MB	3218	1	3217	0	0	0	0	0
Trogoderma variabile	Control	2193	2189	4	247	194	367	253	2196
	EDN	3118	4	3114	0	0	0	0	0
	MB	3261	3	3258	0	0	0	0	0

Table 6. Comparison between *Ct* product of EDN and MB to achieve 100% mortality of five insect species.

Insects and stages	*Ct* product (mg h/L) required to achieve 100% mortality after 6 hours exposure			*Ct* product (mg h/L) required to achieve 100% mortality after 24 hours exposure		
	EDN	MB	**[MB]/[EDN][1]**	EDN	MB	**[MB]/[EDN]**
Lasioderma serricorne						
Adult	25	35	**1.4**	25	30	**1.2**
Pupa	45	82	**1.8**	46	90	**2.0**
Larva	41	105	**2.56**	40	105	**2.63**
Egg	35	120	**3.43**	35	124	**3.54**
Sitophilus oryzae						
Adult	24	42	**1.8**	23	38	**1.7**
Pupa	72	75	**1.04**	75	80	**1.1**
Larva	62	70	**1.1**	62	70	**1.1**
Egg	62	70	**1.13**	68	70	**1.03**
Rhyzopertha dominica						
Adult	18	35	**1.9**	21	45	**2.1**
Pupa	27	51	**1.9**	25	55	**2.2**
Larva	27	49	**1.81**	25	45	**1.80**
Egg	44	49	**1.11**	47	45	**0.96**
Tribolium castaneum						
Adult	22	80	**3.6**	18	75	**4.2**
Pupa	22	96	**4.4**	24	92	**3.8**
Larva	18	90	**5.0**	18	86	**4.8**
Egg	18	90	**5.0**	18	80	**4.4**
Trogoderma variabile						
Adult	30	70	**2.3**	35	69	**2.0**
Pupa	50	90	**1.8**	50	91	**1.8**
Larva	50	80	**1.60**	50	78	**1.56**
Egg	63	80	**1.27**	65	78	**1.20**

5.2 Phase 2 and 3

5.2.1 Penetration of EDN and MB during exposure

Penetration of EDN and MB into three types of tested soft timber (pinewood Radiata, Douglas and Merbau) showed no significant differences, approximately 5-10% variation (Tables 7 and 8 and Figures 14-17 Appendix)

In soft timber, EDN penetrated through to 15 cm at both 20% and 40% fill rates after 24 hours fumigation although the penetration varied with the timber thickness (Tables 7 and 8).

At 10x10x30 cm and a 20% fill rate, EDN penetration decreased by 14% between a depth of 5cm – 15 cm (Table 7) and by 10% when the fill rate was at 40% (Table 8).
This compared to a 40% drop in penetration between 5 – 15 cm penetration at both 20% and 40 % fill rates when the soft timber thickness was increased to 20x20x30 cm (Tables 7 and 8).

The loss of penetration at timber depth was more pronounced with hard timber with a 70% drop in penetration between 5 – 15 cm in 10x10x30 cm hard timber at a 20% fill rate (Table 7) and a 75% drop at a fill rate of 40% (Table 8).

EDN penetration dropped to zero (failed to penetrate to 15cm) when hard timber thickness was increased 20x20x30 cm either at a 20% fill rate (Table 7) or 40% fill rate (Table 8). EDN did penetrate to a depth of 10 cm for this thickness but at relatively low CT values (74 g h/m^3 at 20% fill rate dropping to 35 g h/m^3 at a 40% fill rate).

In general, MB did not perform (penetrate into timber) as well as EDN in the experimental systems.

MB did penetrate to a depth of 15 cm in soft timber for both experimental systems (10x10x30 cm and 20x20x30 cm; Tables 7 and 8).

However MB experienced greater losses in penetrating between 5 – 15 cm than EDN with a 85% drop between 5 – 15 cm at a 20% fill rate for a 10x10x30 cm soft timber block (Table 7) and a 89% at 20x20x30 cm (Table 11); similar results were obtained at a 40% fill rate (Table 8).

MB did not penetrate to a depth of 15 cm in either hard timber blocks of 10x10x30 cm or 20x20x30 cm (Tables 7 and 8).

MB also failed to penetrate in most instances to a depth of 10 cm in hard timber, the exception being a reading of 18 g h/m^3 obtained for a hard timber block of 20x20x30 cm at a 20% fill rate.

Sorption rates of EDN and MB on both soft and hard timber has no significant change when tested with low and high moisture content of timber fumigated at 15 and 25°C. Low levels of HCN (1-2 g/m^3) were measured after 8 hours fumigation with EDN in both 20 and 40% fill rate chambers.

These experiments suggest MB is generally suitable only as a shallow surface treatment (at depths not exceeding 5 cm from the timber surface).

Individual results for the sorption of EDN and MB for the tested range of timbers and fill rates is providing within the Appendix (Figure 14-17 Appendix).

5.2.2 Penetration of EDN and MB cross grain

Tables 9 and 10 show results that both EDN and MB can not penetrate cross 20x20x30 cm hard timber and MB can only penetrate cross 10x10x30 cm soft timber. EDN can penetrate cross 10x10x30 cm soft and hard timber and 20x20x30 cm soft timber at both 20 and 40% fill rate (Table 9 and 10). These results were consistent with those displayed within Tables 11 and 12 which also show that MB has limited penetration through and cross grain of soft and hard timbers.

Table 7. Ct products at different depths in different timber blocks with 20% fill rate after 24 hours fumigation

Timber types	Timber Sizes	Ct product (g h/m^3) at 5cm		Ct product (g h/m^3) at 10cm		Ct product (g h/m^3) at 15cm		Ct product (g h/m^3) at headspace of chamber	
		EDN	MB	EDN	MB	EDN	MB	EDN	MB
Soft timber (Radiata, Douglas and Merbau)	10x10x30cm	505-515	395-405	475-485	280-285	430-440	75-80	600-610	700-710
	20x20x30cm	335-345	260-270	270-280	100-110	195-205	35-40	585-590	840-850
Hard timber	10x20x30cm	329.8	261.4	187.1	0	103.9	0	592.2	789.2
	20x20x30cm	223.1	106.0	74.0	18	0	0	684.9	875.5

Table 8. Ct products at different depths in different timber blocks with 40% fill rate after 24 hours fumigation

Timber types	Timber Sizes	Ct product (g h/m^3) at 5cm		Ct product (g h/m^3) at 10cm		Ct product (g h/m^3) at 15cm		Ct product (g h/m^3) at headspace of chamber	
		EDN	MB	EDN	MB	EDN	MB	EDN	MB
Soft timber (Radiata, Douglas and Merbau)	10x10x30cm	400-410	310-320	380-390	225-230	340-350	75-80	500-510	720-730
	20x20x30cm	330-340	160-170	270-280	75-80	200-210	35-40	450-460	660-670
Hard timber	10x10x30cm	297.6	212.4	149.2	0	77.9	0	502.3	704.1
			50.5	35	0	0	0	540.7	790.3
	20x20x30cm	145.6							

Table 9. Ct products at different depths in different two ends sealed timber blocks with 20% fill rate after 24 hours fumigation

Timber types	Timber Sizes	Ct product (g h/m^3) at 5cm		Ct product (g h/m^3) at 10cm		Ct product (g h/m^3) at 15cm		Ct product (g h/m^3) at headspace of chamber	
		EDN	MB	EDN	MB	EDN	MB	EDN	MB
Soft timber (Radiata, Douglas and Merbau)	10x10x30cm	410-430	75-80	410-430	75-80	400-430	65-70	720	950
	20x20x30cm	205-215	0	195-210	0	200-210	0	760	1010
Hard timber	10x20x30cm	121	0	110	0	105	0	735	1180
	20x20x30cm	0	0	0	0	0	0	790	1250

Table 10. Ct products at different depths in different two ends sealed timber blocks with 40% fill rate after 24 hours fumigation

Timber types	Timber Sizes	Ct product (g h/m^3) at 5cm		Ct product (g h/m^3) at 10cm		Ct product (g h/m^3) at 15cm		Ct product (g h/m^3) at headspace of chamber	
		EDN	MB	EDN	MB	EDN	MB	EDN	MB
Soft timber (Radiata, Douglas and Merbau)	10x10x30cm	425-435	80-90	430-435	80-90	410-420	75-85	1180	1380
	20x20x30cm	220-225	0	210-215	0	220-230	0	1250	1510
Hard timber	10x20x30cm	121	0	110	0	105	0	1360	1710
	20x20x30cm	0	0	0	0	0	0	1450	1800

Table 11. Ct products at different depths in different one end sealed timber blocks with 20% fill rate after 24 hours fumigation

Timber types	Timber sizes	Ct product (g h/m^3) at 5cm		Ct product (g h/m^3) at 10cm		Ct product (g h/m^3) at 15cm		Ct product (g h/m^3) at 20cm		Ct product (g h/m^3) at 25cm		Ct product (g h/m^3) at headspace of chamber	
		EDN	MB	EDN	MB	EDN	MB	EDN	MB	EDN	MB	EDN	MB
Soft timber (Radiata, Douglas and Merbau)	10x10x30cm	510-530	400-410	470-480	280-290	435-440	70-75	400-405	60-65	395-410	55-60	620-650	750-800
	20x20x30cm	330-340	255-265	275-280	110-115	190-200	30-35	185	0	170	0	650-680	890-905
Hard timber	10x10x30cm	330	255	190	0	110	0	105	0	100	0	705	850
	20x20x30cm	225	105	80	15	0	0	0	0	0	0	750	920

Table 12. Ct products at different depths in different one end sealed timber blocks with 40% fill rate after 24 hours fumigation

Timber types	Timber sizes	Ct product (g h/m^3) at 5cm		Ct product (g h/m^3) at 10cm		Ct product (g h/m^3) at 15cm		Ct product (g h/m^3) at 20cm		Ct product (g h/m^3) at 25cm		Ct product (g h/m^3) at headspace of chamber	
		EDN	MB	EDN	MB	EDN	MB					EDN	MB
Soft timber (Radiata, Douglas and Merbau)	10x10x30cm	450-460	315-330	385-395	220-225	400-430	75-80	390-400	55-60	380-385	50-55	600-615	710-715
	20x20x30cm	310-315	165-175	255-265	65-70	200-210	0	195-205	0	185-195	0	510-525	650-665
Hard timber	10x10x30cm	300	210	155	0	65	0	55	0	55	0	735	1180
	20x20x30cm	150	55	0	0	0	0	0	0	0	0	790	1250

5.2.3 Observed toxicity of EDN and MB at low temperatures

The toxicity of EDN and MB to *T. variabile* after exposure periods of 6 and 24 hours at temperatures of 5°C and 15°C and 65% RH are shown in Table 13 and Figures 18 and 19.

Both EDN and MB achieved 100% mortality of *T. variabile* larvae at both 5°C and 15°C (Figures 18 and 19).

However the observed concentration x time (*Ct*) products of EDN needed to deliver 100% mortality against *T. variabile* larvae were lower than that for MB at both 6 and 24 hours fumigation at both temperatures (Table 13). This indicates that EDN is more efficacious than MB for the control of *T.variabile*.

EDN had a comparatively higher toxicity of about 3 times compared to MB at 5ºC and about 2 times at 15ºC (Table 13). The *Ct* products between 6 and 24 hours exposure were not consistent which indicates that there is not a linear relationship for both EDN and MB concentrations (*C*) and *ex*posure time (*t*).

Table 13. Comparison between Ct product of EDN and MB to achieve 100% mortality of *T. variabile* larvae at 5°C and 15ºC for 6 and 24 hours exposure.

	Ct products (g h/m^3) at 5ºC			Ct products (g h/m^3) at 15ºC		
	EDN	MB	MB/EDN	EDN	MB	MB/EDN
6 hours exposure	105	290	2.76	60	105	1.75
24 hours exposure	170	550	3.24	105	230	2.2

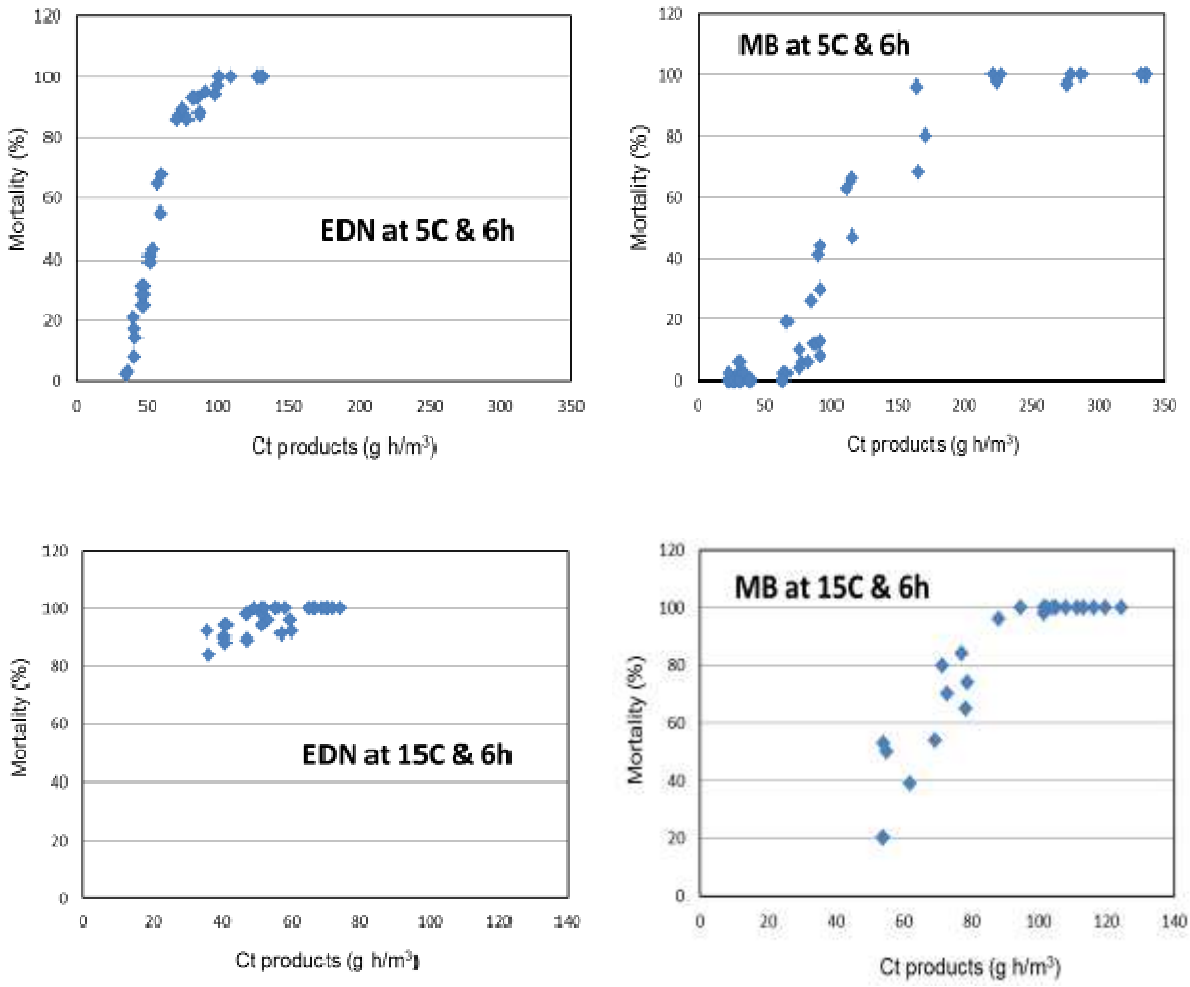

Figure 18. % mortality of *T. variabile* larvae at both 5ºC and 15ºC after 6 hours exposure.

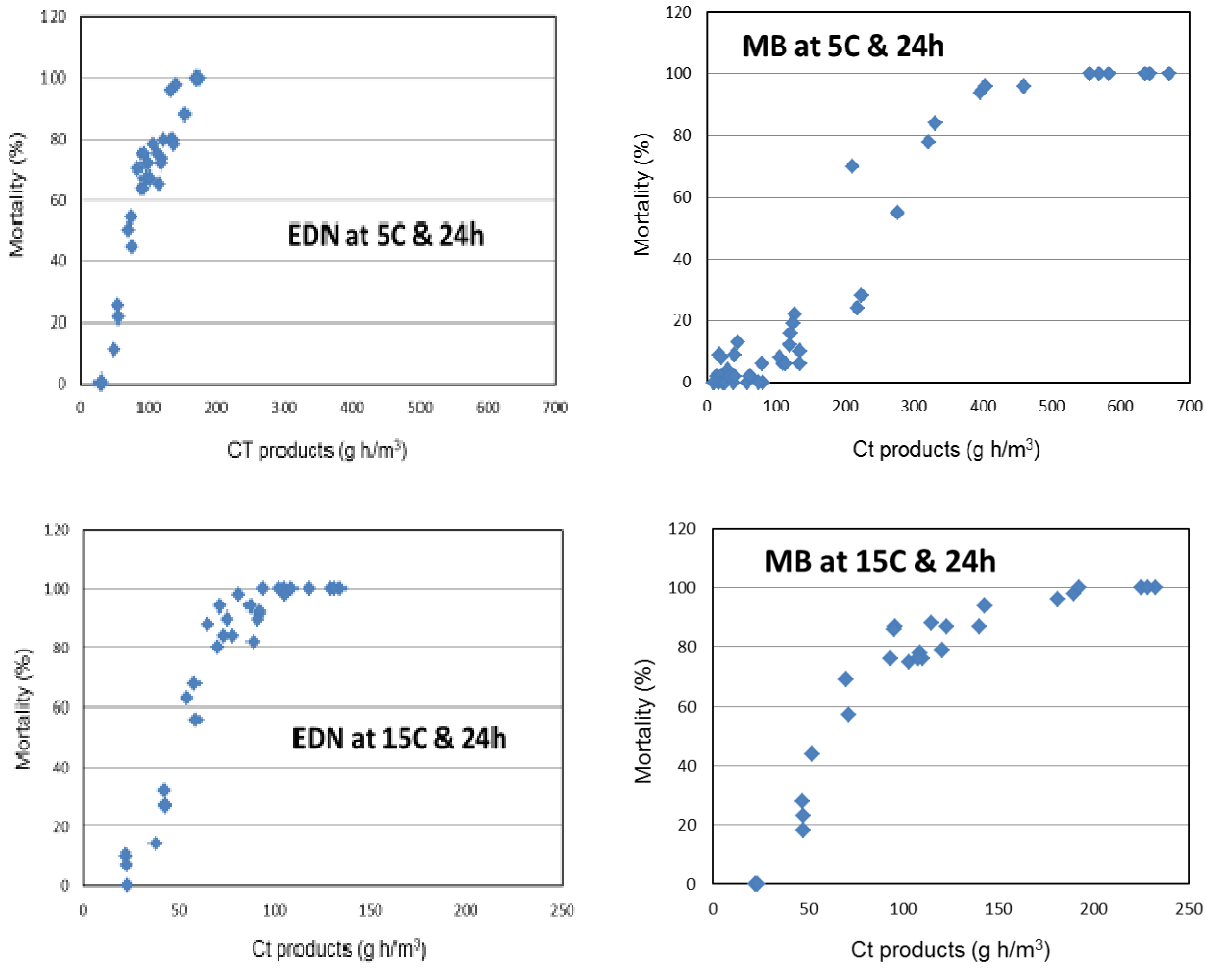

Figure 19. % mortality of *T. variabile* larvae at both 5°C and 15°C after 24 hours exposure.

5.2.4 Observed toxicity of EDN and MB to T. *variabile* larvae and *S. oryzae* pupa in timber block cores

EDN provided control (100% efficacy) of *T. variabile* and *S. oryzae* pupa in both soft and hard timbers to a depth of 15 cm at 15°C and 25°C (Tables 14, 15, 16 and 18). In comparison MB only provided control (100% mortality) to a depth of 5cm in both soft and hard timbers at 15°C and 25°C.

At a depth of 10cm, MB efficacies in soft timbers declined to 75% at a 20% fill rate and 62% at a 40% fill rate. Results at this depth in hard timbers were substantially worse with only 5% efficacy recorded at both fill rates (Table 14). Particularly, at low temperature (15°C), MB can't control *T.variabile* larvae and *S. oryzae* pupae in depths of 10 cm in both soft and hard timbers (Tables 14-15). This latter result is something of an anomaly as MB did not penetrate to this depth (Table 9 and 10). The finding of 5% control may have been due to an artefact of the experimental design with a small amount of MB penetrating along a crack caused by the insertion of the plug.

At a depth of 15cm MB efficacy diminished to 17 – 25% in soft timber; MB provided no control of the beetle in hard timber at this depth.

Mortility of both tested insect have no significant change in tested low and high moisture content of timber fumigated with EDN and MB at 15ºC and 25°C.

Table 14. Laboratory scale fumigation trials in 60 litter fumigation drums loaded with soft and hard timber blocks (20x20x30cm) containing *T. variabile* larvae at 25ºC for 24 hours exposure.

		5 cm		10 cm		15 cm	
		20% fill	40% fill	20% fill	40% fill	20% fill	40% fill
EDN	Soft timber (Radiata)	160/160 100% efficacy	160/160 100% efficacy	160/160 100% efficacy	160/160 100% efficacy	80/80 100% efficacy	80/80 100% efficacy
	Hard timber	160/160 100% efficacy	160/160 100% efficacy	160/160 100% efficacy	160/160 100% efficacy	80/80 100% efficacy	80/80 100% efficacy
MB	Soft timber (Radiata)	160/160 100% efficacy	160/160 100% efficacy	160/120 75% efficacy	160/100 62% efficacy	80/25 25% efficacy	80/14 17% efficacy
	Hard timber	160/160 100% efficacy	160/160 100% efficacy	160/8 5% efficacy	160/8 5% efficacy	80/0 0	80/0 0

Table 15. Laboratory scale fumigation trials in 60 litter fumigation drums loaded with soft and hard timber blocks (20x20x30cm) containing *T. variabile* larvae at 15ºC for 24 hours exposure.

		5 cm		10 cm		15 cm	
		20% fill	40% fill	20% fill	40% fill	20% fill	40% fill
EDN	Soft timber (Radiata)	160/160 100% efficacy	160/160 100% efficacy	160/160 100% efficacy	160/160 100% efficacy	80/80 100% efficacy	80/80 100% efficacy
	Hard timber	160/160 100% efficacy	160/160 100% efficacy	160/160 100% efficacy	160/160 100% efficacy	80/80 100% efficacy	80/80 100% efficacy
MB	Soft timber (Radiata)	160/160 100% efficacy	160/160 100% efficacy	160/51 32% efficacy	160/56 35% efficacy	80/6 7% efficacy	80/4 5% efficacy
	Hard timber	160/160 100% efficacy	160/160 100% efficacy	160/160 0	160/160 0	80/0 0	80/0 0

Table 16. Laboratory scale fumigation trials in 60 litter fumigation drums loaded with soft and hard timber blocks (20x20x30cm) containing *S. oryzae* pupa at 25ºC for 24 hours exposure.

		5 cm		10 cm		15 cm	
		20% fill	40% fill	20% fill	40% fill	20% fill	40% fill
EDN	Soft timber (Radiata)	160/160 100% efficacy	160/160 100% efficacy	160/160 100% efficacy	160/160 100% efficacy	80/80 100% efficacy	80/80 100% efficacy
	Hard timber	160/160 100% efficacy	160/160 100% efficacy	160/160 100% efficacy	160/160 100% efficacy	80/80 100% efficacy	80/80 100% efficacy
MB	Soft timber (Radiata)	160/160 100% efficacy	160/160 100% efficacy	160/74 46% efficacy	160/82 51% efficacy	80/6 8% efficacy	80/9 11% efficacy
	Hard timber	160/160 100% efficacy	160/160 100% efficacy	160/3 2% efficacy	160/10 6% efficacy	80/0 0	80/0 0

Table 17. Laboratory scale fumigation trials in 60 litter fumigation drums loaded with soft and hard timber blocks (20x20x30cm) containing *S. oryzae* pupa at 15ºC for 24 hours exposure.

		5 cm		10 cm		15 cm	
		20% fill	40% fill	20% fill	40% fill	20% fill	40% fill
EDN	Soft timber (Radiata)	160/160 100% efficacy	160/160 100% efficacy	160/160 100% efficacy	160/160 100% efficacy	80/80 100% efficacy	80/80 100% efficacy
	Hard timber	160/160 100% efficacy	160/160 100% efficacy	160/160 100% efficacy	160/160 100% efficacy	80/80 100% efficacy	80/80 100% efficacy
MB	Soft timber (Radiata)	160/160 100% efficacy	160/160 100% efficacy	160/18 11% efficacy	160/24 15% efficacy	80/0 0% efficacy	80/0 0% efficacy
	Hard timber	160/160 100% efficacy	160/160 100% efficacy	160/0 0	160/0 0	80/80 0	80/0 0

6. Conclusions

Based on an agreed protocol, EDN outperformed MB as a quarantine fumigant for the control of all five tested insect species in timber under laboratory controlled experimental conditions.

The key findings from the research were:

- EDN is more toxic than MB to all life stages of the five test insect species under laboratory controlled experimental conditions.
- EDN can penetrate to a depth of 15 cm in soft timber after 24 hours fumigation.
- The amount of EDN present in soft timber diminishes with depth.
- EDN can penetrate to a depth of 15 cm in hard timber after 24 hours fumigation. However this result is dependent on the thickness of the timber; at a timber thickness of 20x20x30 cm EDN penetration is limited to a depth of 10cm.
- MB does not penetrate as well as EDN. MB will penetrate to a depth of 15 cm in soft timber but at a lower concentration compared to EDN; MB does not penetrate beyond a depth of 5 cm in most situations in hard timbers.
- On this basis it is recommended that MB be used only as a shallow surface treatment (at depths not exceeding 5 cm from the timber surface).
- EDN provides better control of *T. variabile* larvae and *S. oryzae* pupa than MB at low temperatures.

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9. Appendix

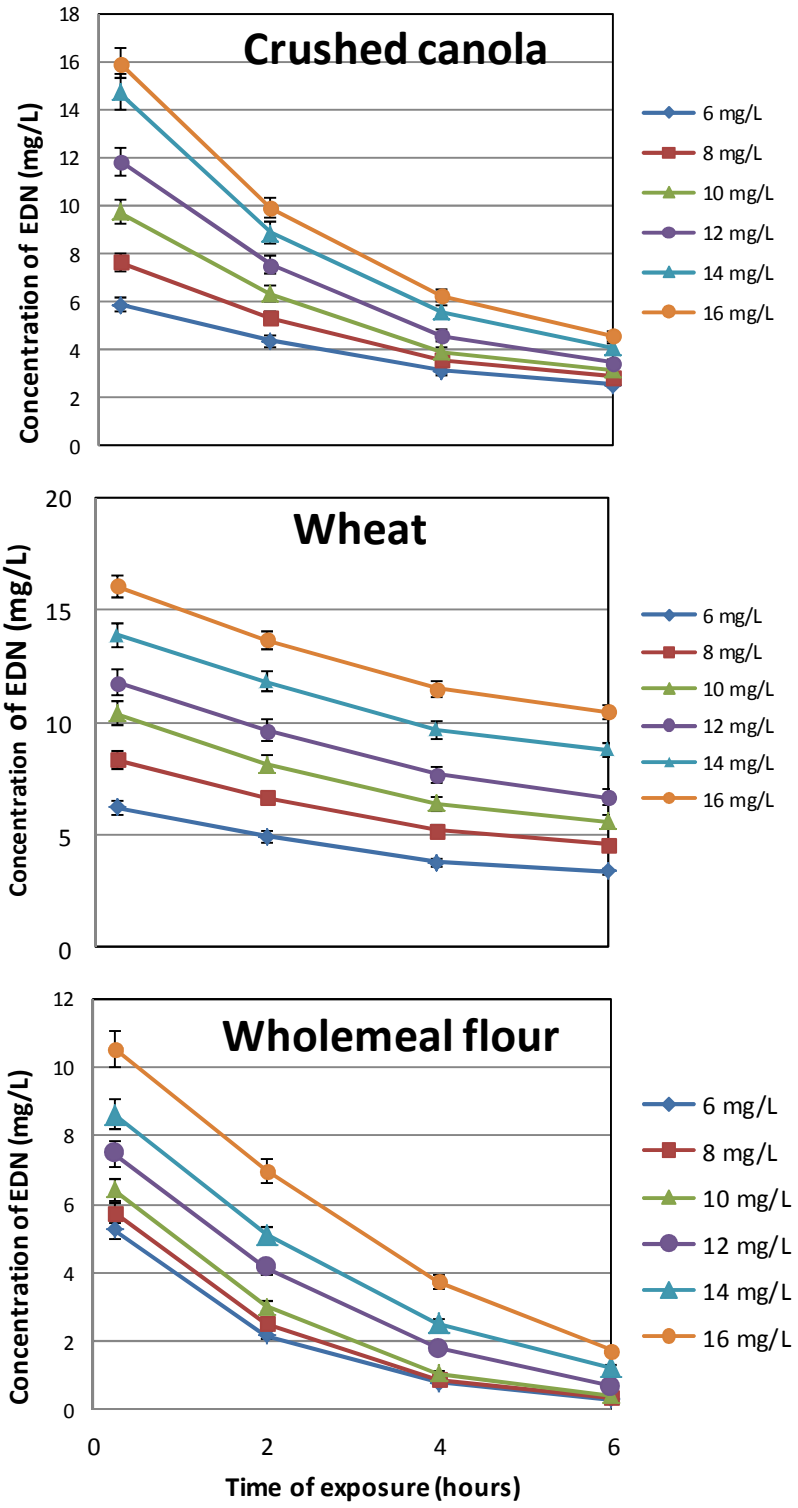

Figure 10. Sorption of EDN on different media (50 g) at different dose rates at 25°C during 6 hours exposure.

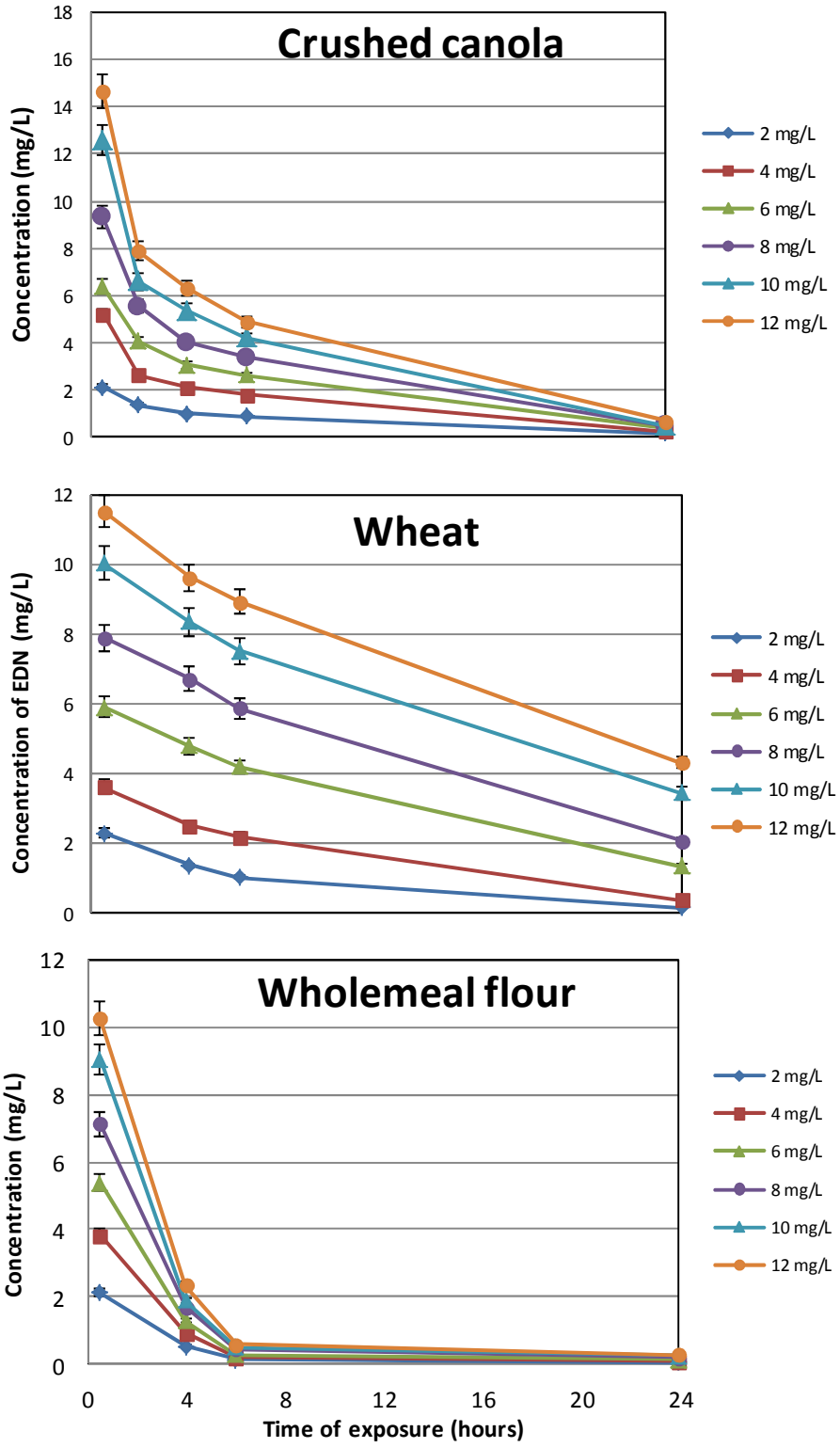

Figure 11. Sorption of EDN on different media (50 g) at different dose rates at 25°C during 24 hours exposure.

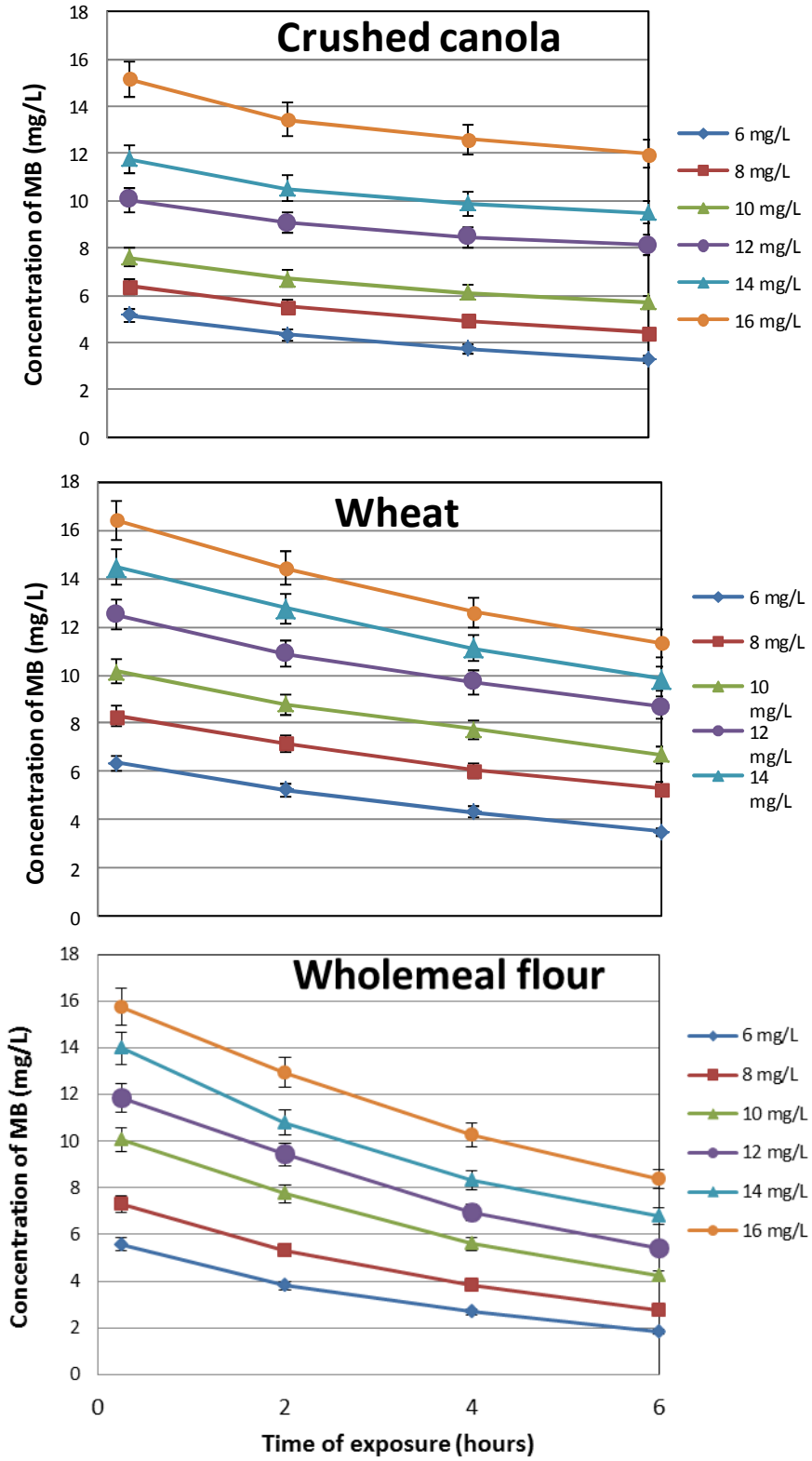

Figure 12. Sorption of MB on different media (50 g) at different dose rates at 25°C during 6 hours exposure.

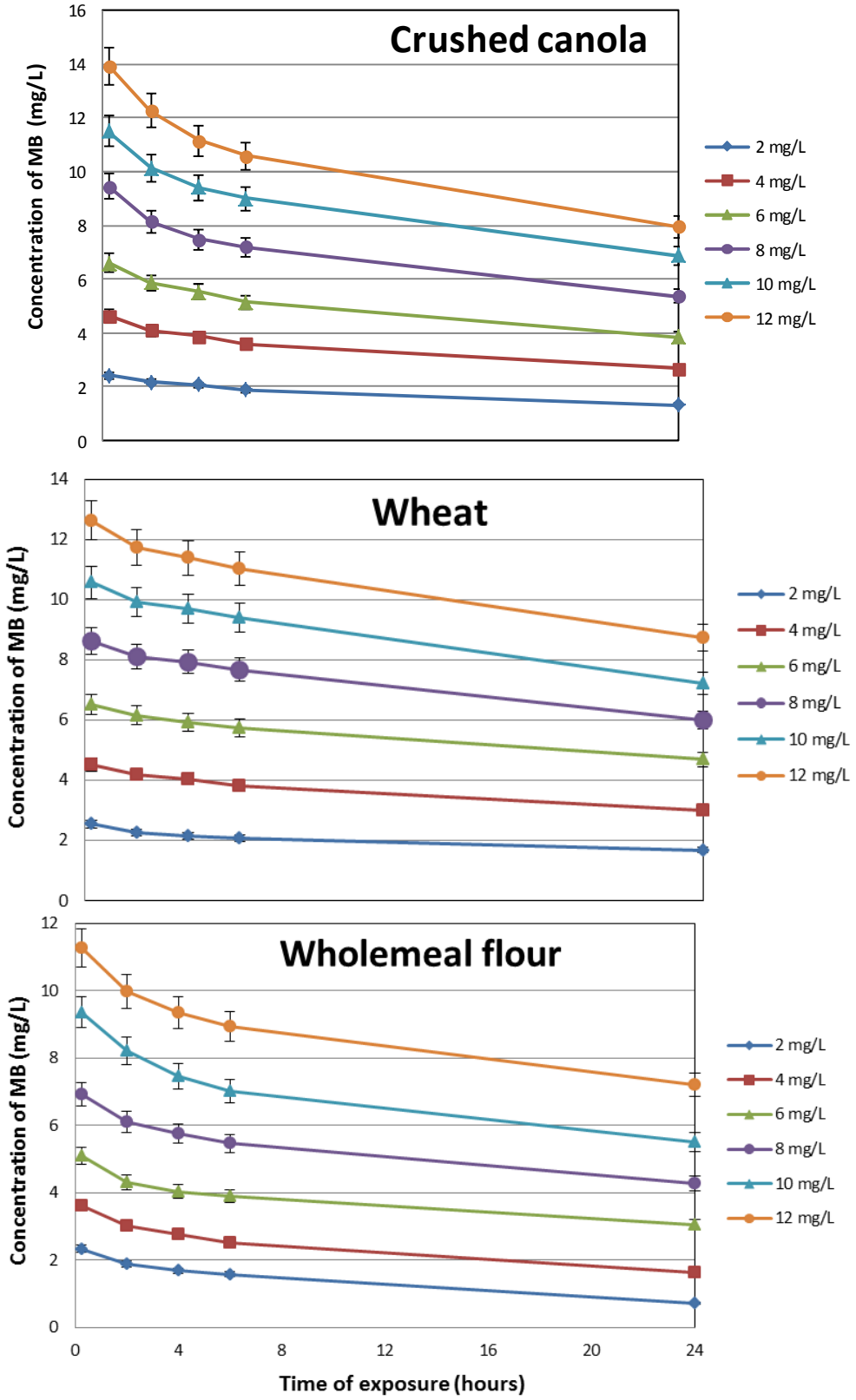

Figure 13. Sorption of MB on different media (50 g) at different dose rates at 25°C during 24 hours exposure.

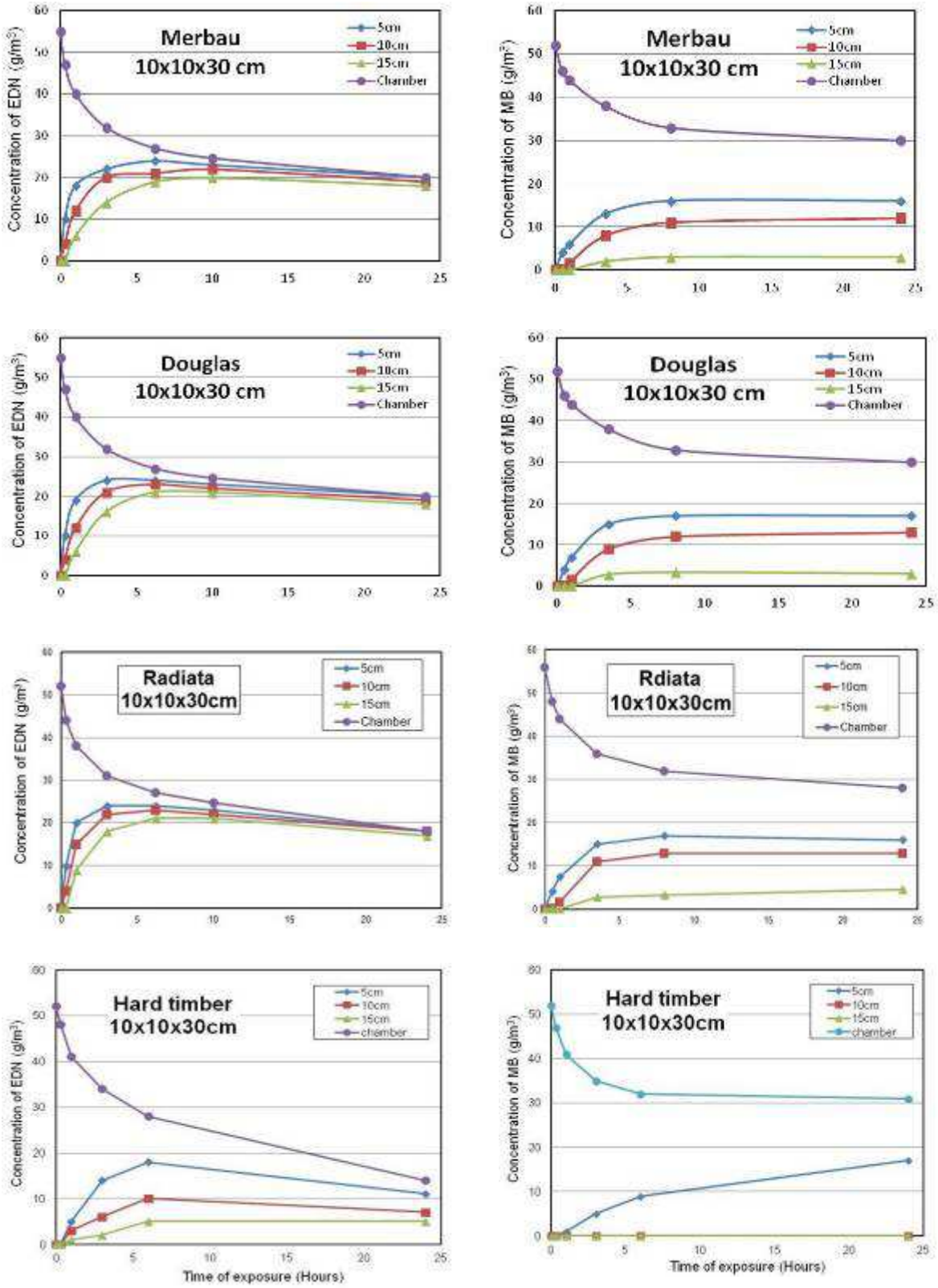

Figure 14. EDN and MB penetration through soft (pinewood: Radiata, Douglas and Merbau) and hard (Jarrah) timbers with timber size of 10x10x30cm and 20% fill rate.

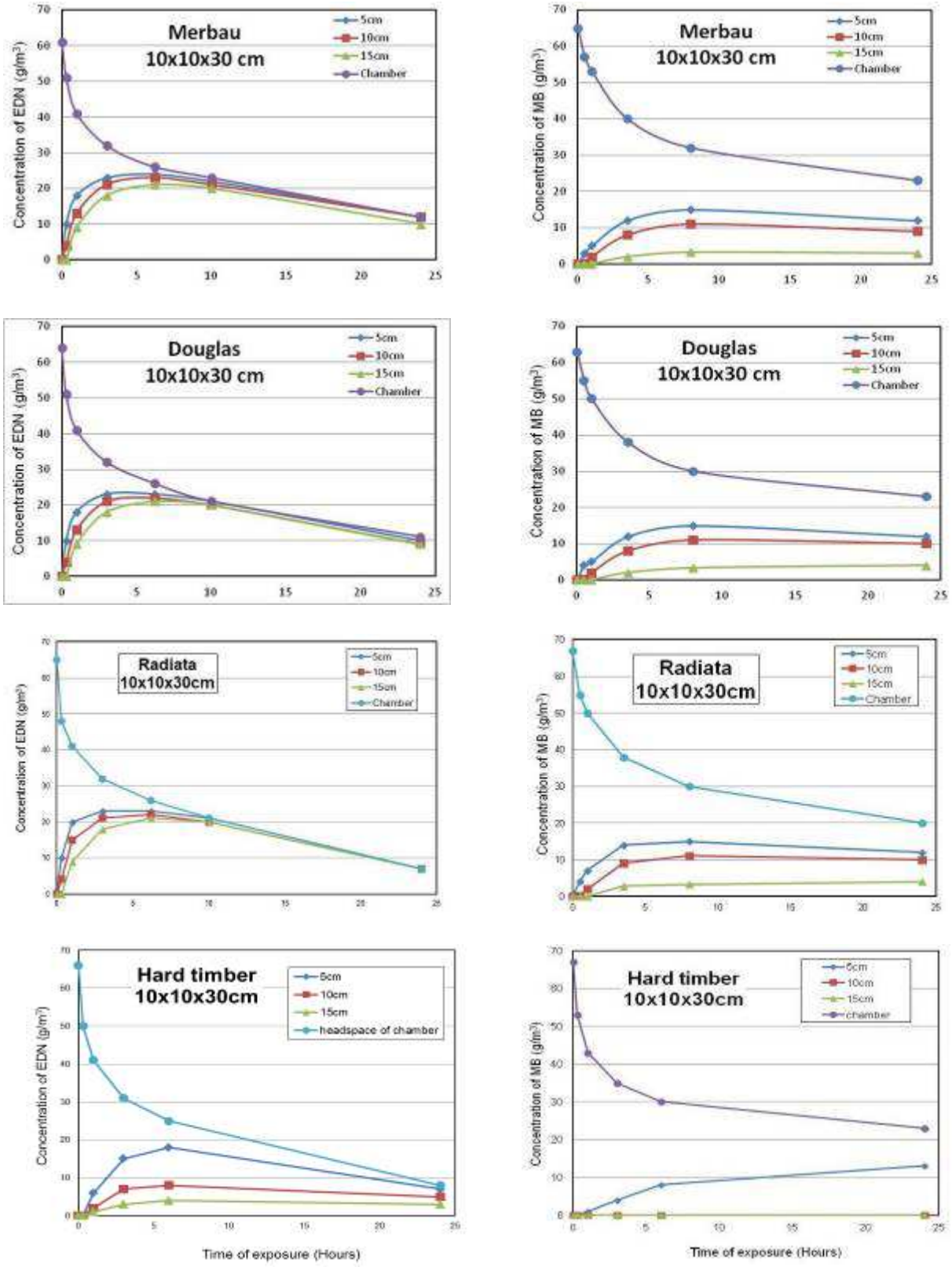

Figure 15. EDN and MB penetration through soft (pinewood: Radiata, Douglas and Merbau) and hard (Jarrah) timbers with timber size of 10x10x30cm and 40% fill rate.

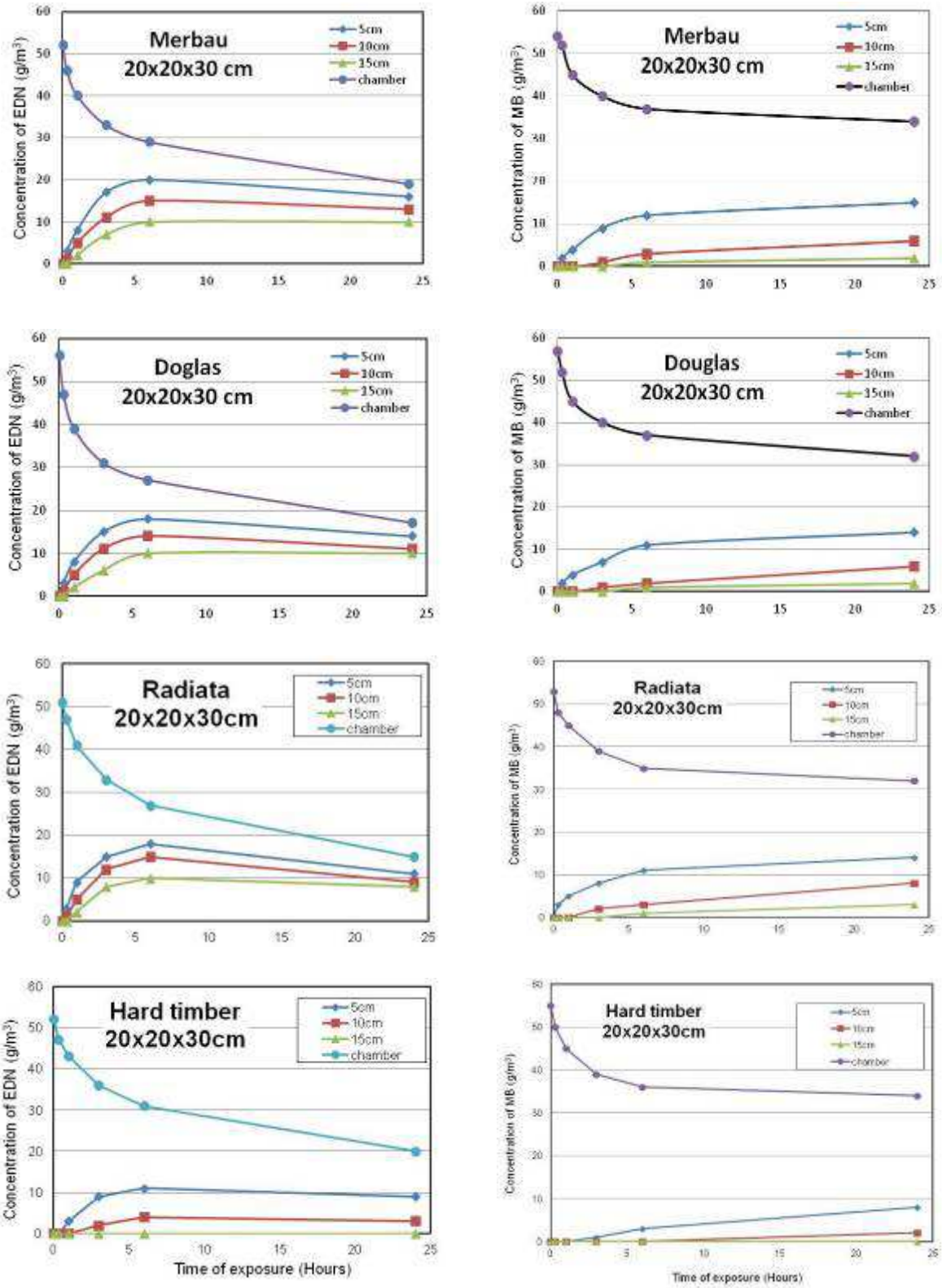

Figure 16. EDN and MB penetration through soft (pinewood: Radiata, Douglas and Merbau) and hard (Jarrah) timbers with timber size of 20x20x30cm and 20% fill rate.

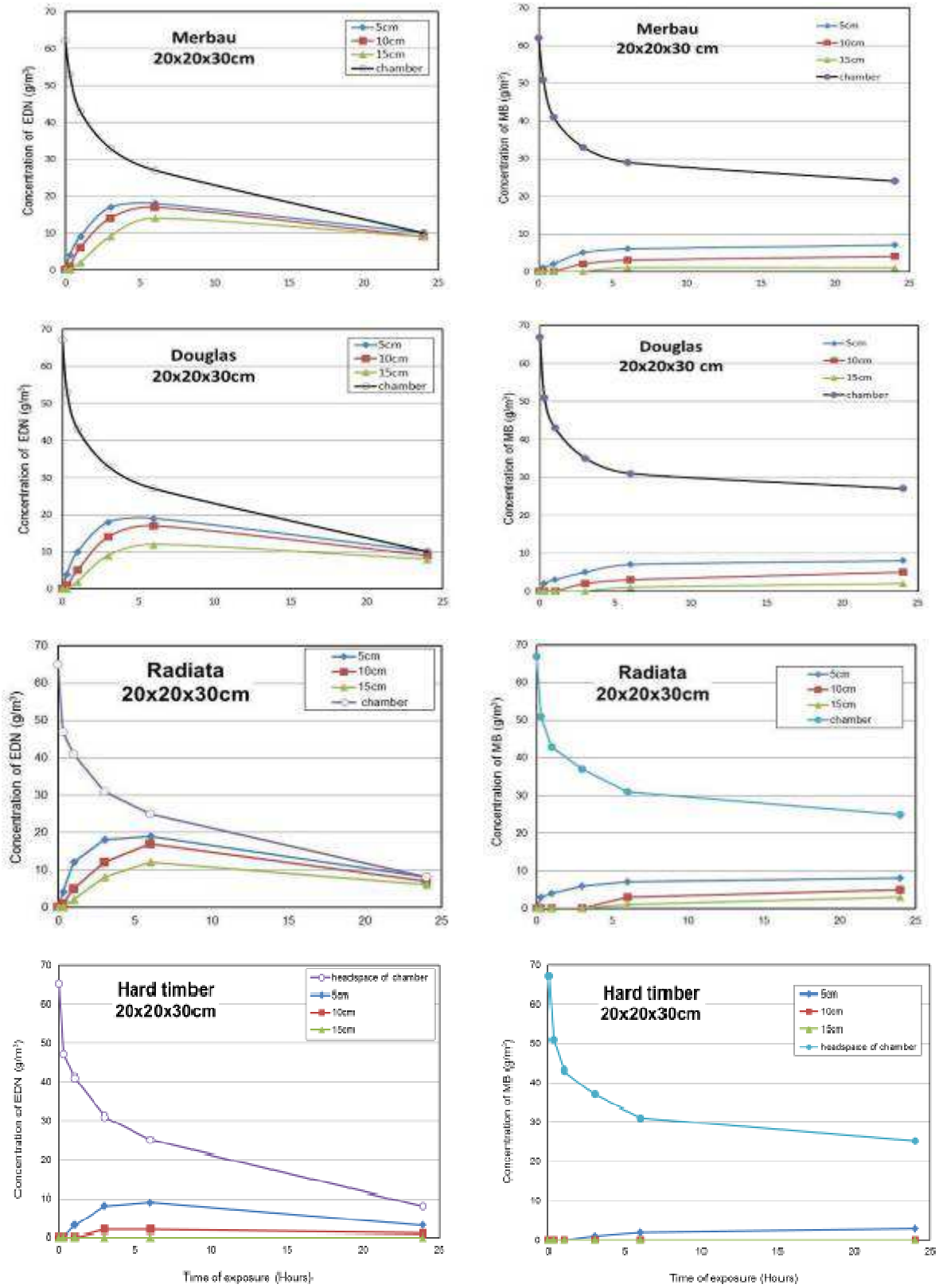

Figure 17. EDN and MB penetration through soft (pinewood: Radiata, Douglas and Merbau) and hard (Jarrah) timbers with timber size of 20x20x30cm and 40% fill rate.