Feasibility of ozone for treating sea containers

(2008 – 10760)

MAF Biosecurity New Zealand Technical Paper No: 2010/01

Prepared for MAFBNZ Operational Research
By Jamieson LE, Wimalaratne SK, Bycroft BL, van Epenhuijsen CW, Page BBC, Somerfield KG, Page-Weir NEM, Chhagan A, Brash DW, Zulhendri F, Plant & Food Research
Rankin D, AgResearch

ISBN 978-0-478-35762-2 (print)
ISBN 978-0-478-35763-9 (online)

ISSN 1179-5832 (Print)
ISSN 1179-5840 (Online)

December 2009

Ministry of Agriculture and Forestry
Te Manatū Ahuwhenua, Ngāherehere

Disclaimer

The information in this report and any accompanying documentation is accurate to the best of the knowledge and belief of Plant & Food Research Limited and AgResearch Limited acting on behalf of the Ministry of Agriculture and Forestry. While Plant & Food Research Limited and AgResearch Limited have exercised all reasonable skill and care in preparation of information in this report, neither Plant & Food Research Limited, AgResearch Limited nor the Ministry of Agriculture and Forestry accept any liability in contract, tort or otherwise for any loss, damage, injury, or expense, whether direct, indirect or consequential, arising out of the provision of information in this report.

Requests for further copies should be directed to:

Strategic Science Team
Policy and Risk Directorate
MAF Biosecurity New Zealand
P O Box 2526
WELLINGTON

Telephone: 0800 00 83 33
Facsimile: 04-894 0300

This publication is also available on the MAF website at
www.biosecurity.govt.nz/about-us/our-publications/technical-papers

Contents

Page

1. Abstract

Currently, shipping containers that arrive in New Zealand and are found to require phytosanitary treatment against invasive pests are fumigated with methyl bromide. There is a concerted effort by scientists and regulatory agencies in many countries, including New Zealand, to develop replacement disinfestation methods that are less toxic to humans and less detrimental to the environment. One Generally Recognised As Safe (GRAS) compound under consideration is ozone gas, which is safer to apply and does not leave persistent residues. This report describes a series of tests to determine the efficacy of ozone against a range of invertebrate pest species that may be associated with shipping containers. Assessment of the effect of ozone on the integrity of a range of materials was also carried out.

The efficacy of a high dose of ozone (10,000ppm) applied over a range of time periods was assessed. Snails were the most tolerant pest tested, requiring an estimated 11.4 hours exposure to 10,000 ppm for 99.9 percent mortality. Mould mites, lightbrown apple moth eggs and lightbrown apple moth pupae were the next most tolerant pests, requiring an estimated 2.9-4.3 hours of exposure. Spiders, beetles, ants, cockroaches and scale insects were controlled in 0.5-2 hours.

The efficacy of a range of relatively lower doses of ozone (between 50 and 2000 ppm) applied over a 24 hour period was also assessed. It was estimated that 274 ppm (138-543 ppm, 95 percent CI) and 1091 ppm (551-2160 ppm, 95 percent CI) for 24 hours was required to kill 99.9 percent of lightbrown apple moth pupae and eggs respectively.

From the materials testing and analysis carried out to date it would appear that appropriately specified shipping container materials may not be affected negatively by high-level ozone disinfestation treatments such as those investigated in this work. However, some types of commonly used plastic packaging materials (i.e. high-density polyethylene, PE-HD) ink and rubber could be negatively affected.

Keywords: ozone, pest mortality, materials testing, tensile strength

2. Introduction

2.1. BACKGROUND

Currently, shipping containers that arrive in New Zealand and found to require phytosanitary (disinfestation) treatment against invasive pests are fumigated with methyl bromide. However, methyl bromide use is being phased out under the Montreal Protocol because it depletes atmospheric ozone (Taylor 1994; Gullino et al. 2005). Furthermore, although phytosanitary uses are presently protected by the Montreal Protocol, methyl bromide may be phased out entirely because of increasing concerns about worker safety and increasing production costs that may make methyl bromide use prohibitively expensive (Taylor 1994; Gullino et al. 2005). There is a concerted effort by scientists and regulatory agencies in many countries, including New Zealand, to develop replacement disinfestation methods that are less toxic to humans and less detrimental to the environment.

Physical methods, such as controlled/modified atmospheres, heat/cold treatments, removal systems, and vacuum/pressurized systems, have shown promise as alternatives to methyl bromide (Dentener et al. 1997; Fuester et al. 2004; Drake et al. 2005; Davenport et al. 2006). However, these physical treatments are often limited by prohibitive energy costs (e.g. heating/cooling systems, pressurized pumps, gas-tight chambers), commodity intolerance to the treatment, or by long exposure times incompatible with standard handling and shipping practices.

Chemical alternatives, such as phosphine, sulphuryl fluoride, carbonyl sulphide, ethyl formate, and ozone, were tested in recent years for disinfestation efficacy against pest species (van Epenhuijsen et al. 2007; Xin et al. 2008). Although phosphine was regarded as one of the more promising candidates and has been widely used, the fumigant has some human safety issues, lengthy fumigation times for efficacy, and can cause the rapid onset of resistance in some pests (Fields & White 2002; Sousa et al. 2008). Sulphuryl fluoride and carbonyl sulphide have been used to disinfest wheat, structures, lumber, wood and artefacts (Bell 2000; Fields & White 2002; Ren et al. 2008). Because these chemicals are highly toxic to humans and there is evidence that edible products absorb these chemicals (residues) (Ren & Mahon 2006), safer alternatives, including GRAS (Generally Recognised As Safe) compounds are being considered. One GRAS compound under consideration is ozone gas, which is safer to apply and does not leave persistent residues (Palou et al. 2003).

2.2. OZONE USES

Ozone has been used as a disinfectant in drinking water since 1893, and as a preservative for cold storage of meats since 1909. In 1939, it was found to prevent growth of yeast and mould during the storage of fruit (Del Agricultural 2000). Ozone has been widely used in marine aquaria, fish disease laboratories, heating and cooling units, water treatment, food processing, bleaching of paper pulp, and in the medical industry to disinfect against microbes and viruses (Weavers & Wickramanayake 2001; Kim et al. 2003). Ozone is also used as a means of reducing odour and for removing taste, colour, and environmental pollutants in industrial applications (Horvath et al. 1985). The chemical and physical properties of ozone are summarised in Appendix 1.

A major advantage ozone has over other chemical treatments used for phytosanitary (disinfestation) purposes is its relatively lower toxicity to humans. In 1982, the US Food and Drug Administration (FDA) listed ozone as a GRAS compound for treatment of bottled water

and the GRAS status was renewed by FDA without change in 1995 (Del Agricultural 2000). In 2001, FDA approved the use of ozone as an antimicrobial agent in food (FDA 2001), again as a GRAS compound. Regardless of the GRAS status of ozone, it is still reactive and has significant health and safety issues (Appendix 2). Therefore, it is recommended that treatment applicators are protected with respirators and treatment facilities are installed with effective scrubbing systems to minimise the effects of ozone on workers and the environment, when working with ozone in large-scale fumigation situations.

2.3. PESTICIDAL ACTIVITY OF OZONE

Ozone is a strong oxidant that exerts oxidative stress on organisms by damaging cell membranes that then impairs the production of ATP (Diao et al. 2004).The use of ozone as a fumigant against arthropod pests has gained increasing interest over the past decade, primarily for control of pests of stored-products. Ozone (with and without vacuum) has been found to be effective against a range of pests including long-tailed mealybugs (*Pseudococcus longispinus* Targioni Tozzetti), western flower thrips (*Frankliniella occidentalis* (Pergande)), coffee berry borer (*Hypothernemus hampei* (Ferrari)), two flour beetles, (*Tribolium confusum* Jacquelin du Val and *Tribolium castaneum* (Herbst)) Indian-meal moth (*Plodia interpunctella* (Hübner)), sawtoothed grain beetle (*Oryzaephilus surinamensis* L.) and the biting gnat (*Culicoides variipennis* (Coquillett)) (Mason et al. 1997; Erdman 1980; Akey 1982; Strait 1998 in Kells et al. 2001; Hollingsworth & Armstrong 2005; Armstrong 2008). Used as a fumigant, efficacious ozone concentrations can vary from < 45 ppm to 10,000 ppm and exposure durations can vary from hours to days (Rajendran 2001; Armstrong 2008) depending on the target organism and fumigation parameters, such as temperature, O_2/CO_2 concentration or vacuum (Hollingsworth & Armstrong 2005; Sousa et al. 2008). A summary of the efficacy of ozone against pests and pathogens is provided in Appendix 3.

Fumigation using high concentration of ozone (10,000 ppm) combined with vacuum (-32 cm Hg) for 6 h has been investigated to control potential infestations of coffee berry borer and coffee leaf rust in green coffee beans imported into Hawaii. This treatment has been shown to kill all life stages of coffee berry borer except eggs (Armstrong 2008).

Pest species that are commonly intercepted in association with sea containers entering New Zealand include ants, moths, snails, beetles, cockroaches, mites and spiders.

2.4. IMPACT OF OZONE ON THE INTEGRITY OF MATERIALS

Because ozone is a strong oxidant that reacts with and degrades alkenes, aromatics, ethers, bromine, nitrogen compounds and rubber. A major disadvantage of ozone is its corrosiveness to most metals (Mason et al. 1999) except stainless steel, gold and platinum (International Chemical Safety Cards 1993). Corrosive activity generally occurs above 2-3 ppm ozone, especially in the presence of moisture (Harvath et al. 1985). However, most studies examined the response of a material to long exposure times (hundreds of hours), whereas a disinfestation treatment against target pests would probably be ≤ 24 h. Materials, such as metal alloys and silicon, are not recommended for use as ozone containers (Shanbhag & Sirkar 1998; Ozen & Floros 2001). Sleeper & Henry (2002) recommended only austenitic grades of stainless steel for ozone treatment vessels (fumigation chambers) because most metals are easily corroded by ozone (Rajendran 2001). Because ozone is a non-penetrating surface fumigant, the only subsurface penetration in solid materials would occur when cracks, fissures, production anomalies, or damage extending into the material are present.

Exposure to ozone will inevitably affect the properties of synthetic materials, especially polymer-based materials that are usually used in fruit packaging. Exposure to ozone was shown to modify the surface properties of polymers and enhance their hydrophilicity and adhesion properties (Mathieson & Bradley 1996; Macmanus et al. 1999). Exposure to ozone can also lead to the formation of oxygen-containing functional groups in the polymers and this consequently degrades them (Anachkov et al. 1993).

Common polymers used for packaging, such as polyethylene (PE), polyethylene tetraphalate (PET), polypropylene (PP), polyaniline (PA), and Polystyrene (PS), exhibit different changes in the properties when exposed to ozone. Most PE-based plastics are susceptible to damage from ozone exposure (Kefeli et al. 1971). Ozen et al. (2002) reported that ozone exposure induced the formation of oxygen-containing functional groups and degradation of the polymeric chain of PE, and ozone consequently impaired the mechanical properties of the polymers. Conversely, ozone treatment increased the tensile strength of PA. They also reported that ozone exposure could reduce oxygen permeability in PE and PA. Ozone exposure can lead to formation of radical cations or polarons in the polymeric chains of PA. Formation of nitrites and nitrates in PA after ozone exposure is not uncommon (Cataldo 2002). PS is the most ozone-sensitive of all the polymers because the aromatic (styrene) rings are very susceptible to ozone attack (Ozen & Floros 2001). For example, Nair et al. (2008) found that exposing styrene butadiene rubber to 0.5 ppm O_3 for 120 h resulted in cracking.

Construction of disinfestation systems should use the following as a guide. Seals suitable for use with ozone are viton, silicon, hypalon, EPDM (although these do wear over time) and Teflon. Nitrile products are not suitable, as ozone quickly degrades rubber. Fittings should be 316SS, with PE (polyethylene) tape. Materials suitable for long-term exposure i.e. tanks, pipes, distribution lines and so on should be high density PVC (certain brands only), PE, Teflon, 316SS or higher grades of stainless steel, ceramic & silicone. Sealants used in construction should be without additives, as ozone will often attack the compounds used to speed up setting times.

When developing ozone fumigation as a potential alternative to methyl bromide for shipping containers, the effects of ozone on metals, polymers, and other material that may be present must be considered.

2.5. AIMS

This ozone research project involved a proof-of-concept investigation to determine:

- The efficacy of a high dose of ozone (10,000 ppm) to representatives of key pests (time mortality response) arriving in to New Zealand in sea containers.
- The efficacy of a range of ozone doses against representatives of fruit pests (Lepidoptera and Hemiptera) that can be found on both imports and exports.
- The impact of a treatment that has high efficacy against the most tolerant species tested, on materials contained in containers and the structural integrity of containers.

3. Methods

3.1. EFFICACY OF OZONE AGAINST PESTS

As a proof of concept the efficacy of a high dose (10,000 ppm) of ozone against representatives of intercepted pests was tested.

3.1.1 Pest preparation and assessment

Insects for fumigation tests were either collected wild or provided from established laboratory colonies as described along with pre-treatment preparation, test methods and post-treatment handling for each test species. Regardless of species, containers of test organisms were covered with paper to prevent desiccation after fumigation and allowed to vent for 24 h at 24°C.

3.1.1.1. Lightbrown apple moth – Epiphyas postvittana (Walker)

Lightbrown apple moth eggs or pupae were obtained from a laboratory colony reared on artificial diet (Clare et al. 1987) at 20 ± 1°C, 70 percent RH, with a photoperiod of 16:8 (L:D) h. Samples of 100 pupae were collected from grid boxes and placed into a Petri dish with folded tissue paper. Eggs were collected by placing plastic sheets into oviposition cages with 30 moth pairs 24 h before collection. Segments of the plastic sheet with approximately 100 eggs were cut out and placed in a Petri dish with folded tissue paper. Pupae and <24 h old eggs were transported overnight to Palmerston North, for treatment the following day. A few hours after arriving in Palmerston North, the lid on each Petri dish was replaced with a new lid that had ≈15 holes (4 mm diameter) to facilitate air exchange during fumigation. Each lid was secured with tape. After treatment, the original lids without holes were replaced and secured with tape.

Treated and untreated lightbrown apple moth pupae and eggs were returned to Auckland for observation after 2 to 3 d holding at 24°C. Each pupa was held with forceps and observed for movement (scored as live) or lack of movement (scored as dead). Live and dead pupae were placed in separate plastic containers (625 ml) lined with tissue paper, the openings covered with gauze. The containers were held at 24°C for 8-10 d to provide adequate time for adults to emerge, and then the numbers of emerged adults were recorded.

The number of lightbrown apple moth eggs on each plastic sheet was recorded and the segments were held at 24°C for 14 days to provide adequate time for the eggs to hatch before recording the numbers of infertile, fertile but unhatched and hatched eggs.

3.1.1.2. Brown garden snail – Helix aspersa (Muller)

Samples of snails (>12 mm diam.) were collected in April 2009 from the wild and held in either 1-litre plastic containers with a hole in the lid and base (60 mm diam. and 45 mm diam., respectively) covered with plastic insect netting to prevent escape, or in a plastic container (650 ml) with a 45-mm hole in the lid and base covered with stainless steel mesh to prevent escape. The containers (40-70 snails each) were held for 5 d at 12°C and high humidity provided by water misting to activate snails. Snails were fed lettuce, cabbage and carrot. After 5 d at 12°C, the snails were held at 18°C for 24 h in low humidity to force aestivation and then held at 22°C until they were treated.

Treated and untreated snails were held in open 1-litre plastic containers that were each placed in a 20-litre plastic bucket containing lettuce on which the snails could feed. Snails were held

at 18°C and high humidity provided by water misting to activate them and were observed for viability at two-day intervals for 21-28 days. Snails that migrated from the 1-litre container to the lettuce were scored as live. At the final assessment, any remaining snails were all of a very light weight and were assessed as dead.

3.1.1.3. Hide beetle - Dermestes maculatus (de Geer)

The Hide beetle colony was established with larvae and adults collected from a colony at Biological Department, Te Papa Museum, in 2008. Larvae and adults were held in 54-litre fish tanks containing cotton wool and newspaper to facilitate their cryptic behaviour and reared on meat bones, dead mice and water.

Small and large larvae (20-30) and adults were placed in 400-ml plastic cylinders 1 d before treatment. Each cylinder contained a small piece of beef for food and crumpled paper towel to facilitate larval burrowing. The ends of each cylinder were closed with metal mesh to facilitate air movement and prevent escape.

Hide beetle larvae and adults were removed from the cylinders one day after treatment and placed on a white tray and probed with forceps to stimulate movement. Insects that did not move were scored as dead.

3.1.1.4. Mould mites – Tyrophagus putrescentiae (Schrank)

Mould mites were collected in November 2008 from infested *Cyrtanthus* sp. tubers and reared in plastic containers at 27°C and >90 percent RH. The mites were reared on a diet of powdered rat food and yeast. Holding conditions were changed to 15°C and 65 percent RH for a few weeks to moderate the high rate of mite reproduction.

Mites used for fumigation tests were transferred from the colony to Petri dishes 24 h before testing, by placing 7 g of mite-infested diet in each of seven Petri dishes containing a piece of moist cotton wool on a piece of plastic to prevent desiccation. Each Petri dish lid had a 60-mm diam. hole covered with fine metal mesh to facilitate air exchange and prevent mites from escaping. The two halves of the Petri dish were sealed with Parafilm® and held at 22^{o}C until the tests were done.

Treated and untreated Petri dishes were held for 24 h at 24°C and covered with a damp paper towel and an inverted plastic container to maintain high humidity to facilitate mite survival. To observe mite survival, the treated diet from each Petri dish was sieved (2-mm mesh) on to four adjoining rectangles (5 cm × 8 cm) drawn on a sheet of A4 paper. The diet from each rectangle was brushed onto the sticky side of a piece (90 mm × 48 mm) of black tape. An aluminium rectangle with four 12-mm diam. randomly situated holes was placed over the treated diet, resulting in samples of treated diet in circles for microscopic observation. The number of live mites in each was recorded. If no live mites were found, the entire tape was observed for movement of live mites to ensure there was no survival. Figure 1 shows the observation method for mite survival.

Figure 1: Preparing mould mite samples for assessment

3.1.1.5. Whitefooted house ant - Technomyrmex albipes (Smith)

An ant colony was established in an aquarium with ants collected from infested wood in April 2009. The sides of the aquarium were covered with black plastic to keep light out and a layer of potting mix at the bottom of the aquarium provided material in which the ants could develop their colony. The ants were fed an egg-honey-agar diet (Queensland Museum 2006) and a 10 percent honey water solution.

Ants (30–100) were collected randomly from the colony 24 h before fumigation tests and placed in a cylinder (72-mm diam.) closed at both ends with fine metal mesh to facilitate air exchange and prevent the ants from escaping. A crumple paper towel was placed in each cylinder to provide the ants with surface area and reduce potential mortality from ants clumping in the cylinder and damaging each other. A glass tube closed with dental roll and filled with a 10 percent sugar water solution to provide the ants with a carbohydrate and water source. The ants in the cylinder were held at 16°C until they were treated.

Observation for ant survival after fumigation was done by removing the ants from each glass tube onto a white surface and touching each ant with a probe. Ants that did not move were scored as dead.

3.1.1.6. Nurseryweb spider - Dolomedes minor (Koch)

Web nests with young spiders of various ages were collected from roadside gorse and grasses in Rotorua in April 2009. The web nests were wound onto sticks and the sticks placed into holes drilled into blocks of wood. The wood blocks with spiders were placed in aquariums and held at 17±1°C. The spiders were fed a 10 percent sugar water solution.

Spiders (40-70) for tests were collected with a vacuum aspirator and placed into 120-ml plastic cylinders with some crumpled paper towel and sugar water in tubes plugged with dental roll. The lid and base of the cylinder had metal mesh to facilitate air exchange and prevent the spiders from escaping.

Spiders were observed for survival 24 h after fumigation by touching each spider with a probe. Spiders that did not move were scored as dead.

3.1.1.7. American cockroach - Periplaneta americana (L.)

Cockroach colonies were maintained in 18-litre plastic containers containing wood shavings to provide surface area for the insects and facilitate reproduction. The cockroaches were fed dog biscuits, carrot and water and held at 29°C and in complete darkness.

The day before fumigation, colonies of cockroaches were cooled to ≈ 5°C to induce torpor and facilitate collection of insects for tests. Immature (≈ 30) and adult (≈ 6) insects were placed in 1-litre plastic cylinders with metal mesh in the lid and base to facilitate air exchange and prevent cockroaches from escaping. Test insects were held at ≈ 5 °C until 3 h before fumigation, then warmed at 24°C to return them to normal activity. Egg cases (ootheca) were detached from females, fumigated, and held for three weeks at 29°C before observing for survival. Hatched immatures were scored as surviving eggs. In the fourth of four test fumigations, 5-10 egg cases (1-2 d old) were added to each treatment.

Treated and untreated immature and adult cockroaches were removed from the cylinders, placed on a white tray and probed with forceps to stimulate movement. Insects that did not move were scored as dead.

3.1.1.8. False Katipo - Steatoda capensis (Hann)

False Katipo adults and egg sacs were collected in March 2009. Individual adults were housed in 150-ml plastic cylinders at 25°C and 65 percent RH. Each cylinder had two mesh-covered holes (10 mm) for ventilation and a dowel placed diagonally inside to facilitate web construction and provide a place for egg sac attachment. Young spiders emerging from the

egg sacs were segregated from adults to prevent cannibalism and placed in separate similar cylinders. Adult spiders were fed flies, moths, a 10 percent sugar solution, and young spiders were fed *Drosophila* spp. and sugar water.

Single adults or young spiderlings (15-30) were transferred to 150-ml cylinders with wire mesh at each end to facilitate air movement and prevent spiders from escaping.

Spiders were observed for survival 48 h after fumigation by touching each spider with a probe. Spiders that did not move were scored as dead.

3.1.1.9. Greedy scale – Hemiberlesia rapax (Comstock)

Greedy scale insects were obtained from PFR laboratory colonies reared on red potatoes and butternut squash at 21 ± 1°C, 65-75 percent RH and L:D 16:8 photoperiod. Crawlers were transferred to uninfested potatoes and held in 36-litre containers at 21°C until the completion of adult development. Infested potatoes were placed in mesh material bags for fumigation to contain the insects.

The treated potatoes were held at 20°C for 7 d before observing for scale survival. To determine survival, the waxy cap of each scale was removed and physical characteristics of the immatures and adults were observed under magnification. Viability and mortality were each separated into four classifications: live (body orange/yellow in colour and turgid, mouthparts difficult to remove); live but moribund (body discoloured/browning but turgid); dead but moribund (discoloured and slightly flaccid); and dead (discoloured, flaccid or dry, and mouthparts easy to remove).

3.1.2 Ozone delivery and monitoring

Ozone levels were delivered and monitored throughout the treatment using an automated delivery system (Figure 2). The ozone generator was an OZAT Ozone Generator, Type CFS-1…3A (Ozonia Ltd, Sterrbachstrasse 1, Duebendorf, Germany). The ozone monitor was an Ozone Monitor Enviro, Series IN 2000 Single and multi-channel gaseous analyser (AFX Instrumentation, USA Inc, Needham, MA, USA) and had an internal self-calibrating capability. The monitor was calibrated against a new factory-calibrated ozone monitor before delivery and after completion of fumigation tests (no drift was reported).

The computer software used to enter the instruction into the generator was a customised program build from In Touch for windows V 1.5©. Once the ozone levels and duration of the treatments were instructed by the computer software, instrument grade O_2 was fed into the ozone generator and ozone was generated. Thereafter, specified levels of ozone were injected into each chamber automatically. The levels were maintained by drawing a sample from each chamber (one at a time) and monitoring it through the ozone monitor. If the levels were lower than specified, ozone was injected into the chamber automatically. Ozone concentration was logged every 10 minutes for experiment 1 and every minute for fumigation experiments 2-7.

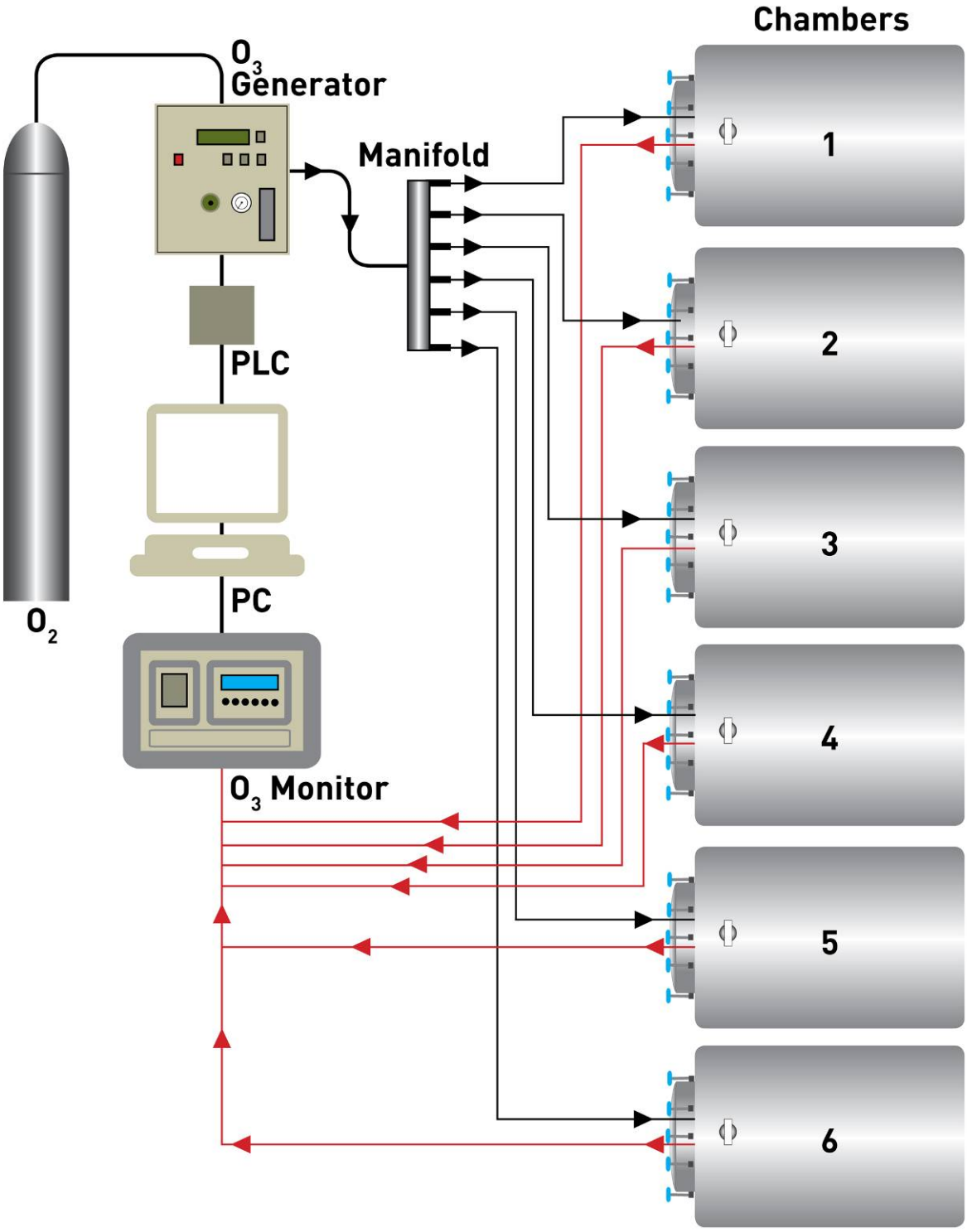


Figure 2: Schematic diagram of the automated ozone delivery system

3.1.3 Treatments

Fumigations were done using two different sets of parameters:

1. four replications with each test invertebrate using 10,000 ppm ozone for 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 8.0, or 16.0 h as a proof of concept demonstrating that ozone can control a wide range of invertebrate pests
2. three replications with each test organism using 50, 100, 500, 1000, or 2000 ppm for 24 h at 7ºC to determine the mortality response of two fruit infesting pests to a range of doses.

Control organisms were handled identically to the treated organisms, except that they were not fumigated. After fumigation chambers were aerated for 30 minutes before test invertebrates were removed.

Figure 3: Chambers used for ozone experiments

3.1.4 Statistical analysis

For mortality response figures (Figures 4 and 5), the loess smoothing function (Chambers & Hastie 1992) was used in R (R Development Core Team 2009) to draw a smooth line through the mean mortality points for each pest after exposure to different ozone treatments at each exposure time or dose. An arcsin transformation (arcsin(sqrt(p))) was applied to the percentage (p) to stabilize the variance (i.e. so that the error bar is appropriated over the entire range of 1-100 percent). Standard errors for each treatment were calculated at every treatment time or dose. The root mean square of these SEMs gave a mean SEM for each life stage at each storage temperature.

For Tables 3 and 6, time mortality data for each replicate were fitted using the complementary log-log (clog-log) model (Preisler & Robertson 1989), with time or dose as the explanatory variable to derive estimated lethal times (days) to achieve 99.9 percent mortality ($LT_{99.9}$). These estimates were calculated as the time to achieve a mortality of $c + (1 - c) \times m$, where c was the control mortality and m the estimated proportion mortality. For each pest and life stage, a geometric mean LT and its associated standard error (SEM) were estimated, from which a 95 percent confidence interval (CI) was calculated. Non-overlap of the 95 percent CIs is equivalent to a test for difference at $P = 0.01$.

3.2. IMPACT OF HIGH CONCENTRATIONS OF OZONE ON THE INTEGRITY OF SELECTED MATERIALS

3.2.1 Selection of materials for testing

Materials evaluated and tests performed are summarised in Table 7.

Table 7. Plastics, rubber, cardboard and metal materials exposed to potential ozone disinfestation treatment.

No.	Type[a]	Description	Supplier	Testing[b]
1	PET 12µm (1)	Polyethylene terephthalate – clear film	Aperio Flexipac, Christchurch	TS
2	PE-HD 20µm (2)	High-density polyethylene – white film	Elldex Packaging Group, Christchurch	TS
3	PVC (3)	Polyvinylchloride	Aperio Flexipac, Christchurch	TS
4	PE-LD 30µm (4)	Low-density polyethylene – clear film	Elldex Packaging Group, Christchurch	TS
5	PP 19µm (5)	Polypropylene – biaxially oriented transparent pp film, ExxonMobil Bicor MB440	Aperio Flexipac, Christchurch	TS
6	PS (6)	Polystyrene – biaxial sheet translucent	Croxley Stationery Ltd, Auckland	TS
7	Nylon 8-10µm (7)	Nylon	Aperio Flexipac, Christchurch	TS
8	Natural rubber	High-grade 60 Shore A hardness (meets BS 1154:2003)	Skellerup Industries Ltd, Christchurch	TS
11	EPDM syn-thetic rubber	Roofing membrane, 1mm thick	Skellerup Industries Ltd, Christchurch	TS
12	Corrugated cardboard	Storage Box, Homewrap Packaging and Supplies Pty Ltd	MAFBNZ, Auckland	BS
13	Corrugated cardboard	Multicoloured Office Max photocopier paper box, plastic coated	MAFBNZ, Auckland	BS
UC1-UC3	Steel	Uncoated/unpainted container steel	MAFBNZ, Auckland	ME
C1-C3	Steel	Coated/painted container steel	MAFBNZ, Auckland	ME, CHTT

[a] Values in parentheses indicate plastic type

[b] TS – tensile strength; BS – burst strength; ME – microscopic examination; CHTT – cross hatch tape test

Plastic films typical of those used in packaging products and representatives of the classes of different plastics were sourced from a variety of companies, who kindly supplied samples free of charge (Table 1). The thin film materials were chosen for testing as they could probably indicate whether damage or physical changes occurred during ozone exposure and because they most closely reflected some of the packaging materials that might be present in container loads of goods. More solid samples of the same materials, such as plastic from storage containers or thicker sheets, were not used in the evaluations. Such materials might only be affected at their surface and bulk material properties might not be affected, thus making quantification of impacts from ozone treatment difficult if not impossible to determine.

Two printed corrugated cardboard boxing samples, with and without a finishing polymer film coating, were supplied by MAFBNZ.

Two rubber samples were generously supplied by Skellerup Industries Ltd [2]. The samples were precut into dumbbells with a nominal 6-mm width for testing. The rubber samples comprised:

- A 2-mm thick black high-grade 60 Shore A hardness natural rubber-based compound meeting the requirements of BS 1154:2003 – Natural Rubber Compounds, which contains suitable antiozonants for optimum protection for natural rubber compounds
- An ethylene propylene diene (EPDM)-based black roofing material, 1 mm thick, a commercially available synthetic rubber roofing product with a saturated backbone with excellent resistance to the effects of ozone.

The first rubber sample was 100 percent natural rubber filled with carbon black and has high tensile strength and good ageing properties and is used in duties such as sealing gaskets, pipe joint rings and moulding applications. The second rubber is specifically designed for very high ozone resistance. It contains 100 percent EPDM-based rubber and is used in duties such as container door seals, channel strips, solar heating tubing, roofing, and steam hoses.

Two sets of container metal samples were supplied by MAFBNZ. The materials were identified as in Table 8. Three samples were cut-off, painted, used-container metal, the interior finishes of which were coated with a thick, grey, very hard, semi-gloss paint, and which were labelled 'inside'. The types of paint system applied were not identified. Two of the painted pieces (C2, C3) were from different parts of the same container and one was from a different container.

Table 8: Steel container samples for ozone exposure.

Sample ID	Description
UC1	180 x 200 mm, uncoated metal panel, grey, very minor surface rust
UC2	175 x 325 mm, uncoated metal panel, grey and black spotted surface, light surface rust
UC3	175 x 395 mm, uncoated metal panel, grey and black spotted surface, light surface rust
C1	185 x 210 mm, coated red on one side, grey on other side labelled 'inside' as received. Heavy corrosion present on outside, light surface rust on inside
C2	170 x 200 mm, grey coated metal panel. One side labelled 'inside' as received. Heavy corrosion on the outside
C3	220 x 295 mm, grey coated metal panel. One side labelled 'inside' as received. Heavy corrosion on the outside

Three pieces of unpainted container steel were from stock repair steel. Samples were cut in half with a band saw, with one half of each sample being submitted to ozone treatment.

3.2.2 Ozone Treatment

Three separate sets of plastic film, rubber, container steel and cardboard packaging materials were treated in three separate laboratory ozone treatment chambers for 11.3 hours (the lethal time calculated to kill 99.9 percent of snails) at 15°C and an ozone concentration of 10,000 ppm on 22 June 2009 at PFR, Palmerston North. Samples were suspended in the chambers so that ozone had free access to all surfaces (Figure 6). Ozone profiles for the three treatment vessels during the treatments are presented in Figure 7.

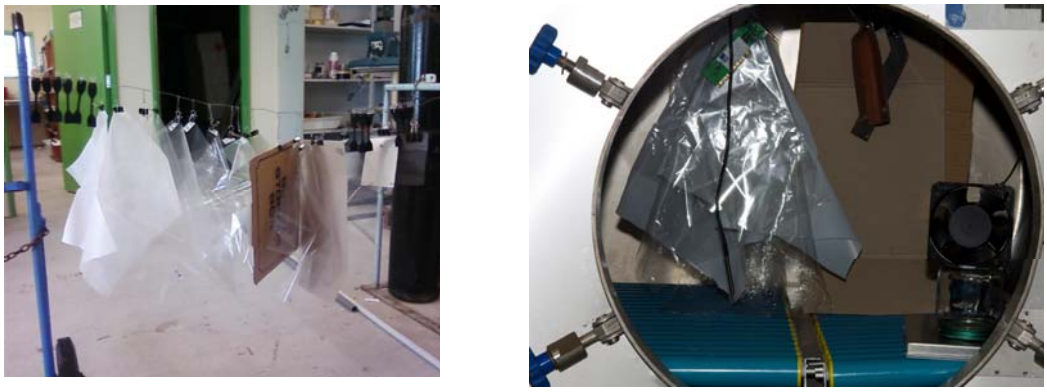


Figure 6: Materials tested for impact of ozone treatment of 10,000 ppm for 11.3 hours before loading in to chambers (left) and after loading in to chambers

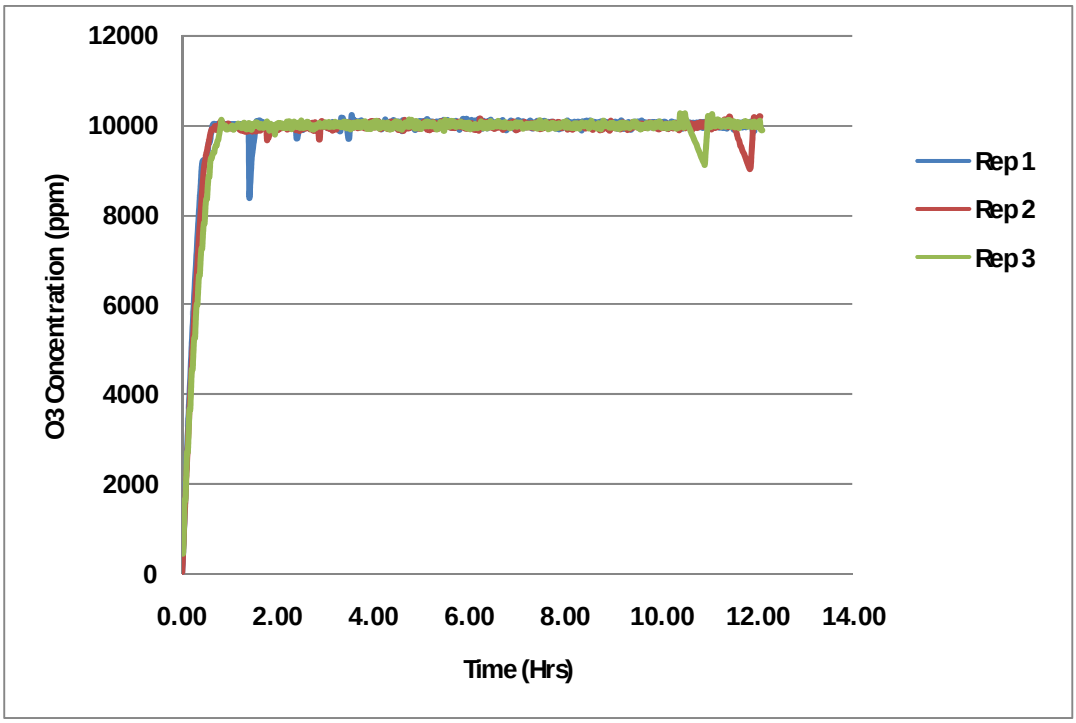


Figure 7: Ozone profiles for the three treatment vessels.

3.2.3 Testing Methods

3.2.3.1. Tensile Testing

Treated and untreated plastic films were measured in triplicate for tensile strength using the 'Standard Test Method for Tensile Properties of Thin Plastic Sheeting' ASTM D 882 – 02, testing samples with their long axes parallel with and normal to the direction of manufacture to determine any anisotropy, after appropriate conditioning at 23±2°C and 50±5 percent RH. Five specimens, one set normal and another set parallel with the principal axis of anisotropy (or the length of the films), were tested from each treated and untreated sample. Sample dumbbells (AS 2282.6 - 1999) were cut by hand with sharp scissors and had a width of 13 mm and an overall length of 152 mm. The distance between the grips on the Instron testing instrument was 100 mm. The breaking factor (maximum load divided by minimum width of specimen), nominal tensile strength (maximum load divided by minimum cross sectional area), nominal tensile strength at break (load at break divided by minimum cross sectional area), percent elongation at break (extension at moment of rupture divided by initial gauge length x 100) and tensile energy to break (integrated total energy per unit volume under the

stress strain curve) were calculated for all samples. Overall mean film thicknesses for respective samples were used when calculating these parameters. Statistically significant differences between parameters from the different treatments were determined using analysis of variance (ANOVA) and Tukey's multiple mean comparison method at the 5 percent level, using statistical software Minitab Version 15.

Treated and untreated rubber dumbbells were measured for tensile strength and breaking elongation using the 'Standard Test Methods for Vulcanised Rubber and Thermoplastic Elastomers-Tension' ASTM D 412 – 98a [4], testing samples parallel to their direction of flow in the mould during manufacture, after appropriate conditioning at 23±2°C and 50±5 percent RH. Each sample was measured in triplicate and three specimens were tested from each treated and untreated sample. Sample dumbbells (Die C) cut by press had a width of 6 mm. The distance between the grips on the Instron testing instrument was 80 mm. Tests were conducted in triplicate and results examined for statistically significant differences using ANOVA and Tukey's multiple mean comparison method at the 5 percent level, using statistical software Minitab Version 15.

3.2.3.2. Burst Strength

Treated and untreated cardboard samples were measured for burst strength using a Perkins Jumbo Mullen Tester using a method similar to that for measuring the bursting strength of paperboard and linerboard [5]. Tests were conducted in triplicate, with 10 tests conducted on each subsample, and results examined for statistically significant differences using ANOVA and Tukey's multiple mean comparison method at the 5 percent level, using statistical software Minitab Version 15.

3.2.3.3. Container Steel Testing

Visual inspection

Initially a visual inspection by eye was carried out on the control samples, with a comparison made to the samples exposed to ozone. An inspection of colour change was subjectively made by eye between juxtaposed control and exposed samples on an area isolated by a grey border placed on top.

Microscope inspection

Following the visual examination by eye, an investigation of the surface was made using an optical microscope. The entire surface was visually scanned at 6x magnification. Microscope images were captured using a Pixera PVC 100C digital camera and a Wild M650 microscope at 16X and 40X magnification. Additional lighting, originating from the left hand side of the samples, was provided to accentuate any surface features.

Crosshatch tape test

A crosshatch adhesion test was carried out, to ASTM D3359-97, "Standard Test Methods for Measuring Adhesion by Tape Test", on randomly chosen areas of the surfaces of unexposed control and ozone exposed samples of the painted panels. As this method assesses the adhesion of a coating film to a metal substrate, this test was not carried out on the surface of the unpainted control panels.

A crosshatch of scribe lines with line spacing of approximately 1 mm was cut into the painted surface using a sharp scalpel. Any loosened paint fragments were removed by brushing lightly with a paintbrush. A length of 3M Scotch Magic Tape (19 mm wide) was placed on top of the crosshatch and pressed firmly down using 20 rubs with an eraser. After 60 seconds, the tape was carefully removed to the edge of the crosshatch pattern, and then peeled swiftly from the crosshatched paint surface by pulling at an angle of 180º.

Comparisons between the control and exposed samples were made of the remaining crosshatch patterns and of the paint removed by the tape. A grade was allocated to the samples by comparison to images provided in the standard. The results of this test are in Table 19 and are referred to as the 'B' grades, as defined in the standard.

X cut tape test

When cutting the crosshatch patterns into the painted surface of sample C1, some of the square sections of paint between the scribed lines flaked from the substrate. Measurement of the thickness of the paint flakes using a micrometer revealed the thickness to be approximately 300 μm. ASTM D3359 suggests that the X Cut tape test be applied to paint film with a thickness above 125 μm. The X Cut tape test was therefore carried out on all three of the painted samples.

For this test, two cuts were made into the painted surface in an X configuration with a smaller angle of approximately 40º. A length of 3M Scotch Magic Tape (19 mm wide) was placed on top of the X cut in the direction of the smaller angle of the X and pressed firmly down using 20 rubs with an eraser. After 60 seconds, the tape was carefully removed to the edge of the X cut and then peeled swiftly from the X cut paint surface by pulling at an angle of 180º.

Comparisons between the control and exposed samples were made of the remaining X cuts and of the paint removed by the tape. A grade was allocated to the samples by comparison to descriptions provided in the standard. The results of this test are in Table 19 and are referred to as the 'A' grades.

4. Results

4.1. EFFICACY OF OZONE AGAINST PESTS

4.1.1 Response to duration of application of a high dose (10,000 ppm) of ozone at 15°C

4.1.1.1. Ozone concentration and temperature (Experiments 1-4)

The fumigation parameters for each experiment are summarised in Table 1. Complete ozone and temperature profiles for each fumigation run (i.e. experiment) are shown in Appendices 4 and 5. The desired 10,000 ppm ozone concentration (± 5 percent) was achieved within 9-20 minutes, with an ozone concentration that averaged between 10,068 ppm and 10,250 ppm thereafter. Temperature during fumigation averaged 14.9-15.3°C.

Table 1: The fumigation parameters for fumigation experiments at 10,000 ppm ozone for selected times

	Date	5/05/2009	12/05/2009	13/05/2009	14/05/2009
	Experiment #	1	2	3	4
	Chambers	All	All	All	All
Ozone	Target (ppm)	10000	10000	10000	10000
	Ramp up[1] (min)	10-20	11-13	9-14	9-11
	Mean ± SEM (ppm)	10068 ± 6	10137 ± 5	10250 ± 19	10149 ± 6
	Min (ppm)	9969	9796	9691	9796
	Max (ppm)	10550	11644	14784	12349
Temp	Target (°C)	15	15	15	15
	Mean ± SEM (°C)	15.2 ± 0.1	14.9 ± 0.02	15.1 ± 0.02	15.3 ± 0.03
	Min (°C)	10.5	12.1	13.95	14.3
	Max (°C)	17	15.7	16.4	16.8

[1] Time to establish ozone concentration within 5% of desired level

4.1.1.2. *Efficacy against pests*

The mean percentage mortalities and total numbers tested of lightbrown apple moth, brown garden snail, hide beetle, mould mites, whitefooted house ant, nurseryweb spiderlings, cockroach, false katipo spiderlings and greedy scale insect are shown in Table 2.

The most susceptible pests were whitefooted house ant soldiers, nurseryweb spiderlings, false katipo spiderlings and greedy scale insects. These organisms were killed after 0.5 h (1 replicate) or 1.0 h exposure to 10,000 ppm ozone.

Table 1: Mean percent mortalities and number of pests tested at 10,000 ppm ozone for 0-16 hours

Pest, life stage	Duration	Replicates	Mean % mortality	SEM	n1
Lightbrown apple moth eggs	Control	4	60.7	4.11	1599
	0.5	1	97.3	-	548
	1	4	94.1	2.16	1848
	1.5	3	96.5	1.25	1303
	2	4	98.4	1.04	1508
	3	3	99.8	0.21	1275
	4	4	100	0	1379
	8	4	100	0	1169
	16	1	100	-	480
Lightbrown apple moth pupal mortality 2-3 days after treatment	Control	4	0.8	0.48	400
	0.5	1	12.0	-	100
	1	4	49.0	3.76	400
	1.5	3	80.4	3.09	301
	2	4	91.8	3.84	392
	3	3	98.7	0.88	300
	4	4	100	0	397
	8	4	100	0	395
	16	1	100	-	101
Lightbrown apple moth adult emergence from treated pupae 8-10 days after treatment	Control	4	8.7	3.06	355
	0.5	1	100	0	100
	1	4	100	0	398
	1.5	3	100	0	301
	2	4	100	0	400
	3	3	100	0	300
	4	4	100	0	397
	8	4	100	0	395
	16	1	100	0	101
Brown garden snails	Control	4	12.2	4.88	217
	0.5	1	18.0	-	39
	1	4	26.9	8.33	197
	1.5	3	52.0	11.49	167
	2	4	45.4	9.75	193
	3	3	76.0	3.29	154
	4	4	69.1	4.84	191
	8	4	92.8	1.93	211
	16	1	100	-	38
Hide beetles (small larvae)	Control	3	0	0.00	71
	1	3	98.5	1.52	63
	1.5	3	100	0	65
	2	3	100	0	62
	3	3	100	0	59
	4	3	100	0	59
	8	3	100	0	60
Hide beetles (big larvae)	Control	4	1.5	0.87	131
	0.5	1	17.1	-	35
	1	4	79.1	13.74	120
	1.5	3	98.9	1.11	90
	2	4	99.2	0.83	128
	3	3	100	0	89
	4	4	100	0	124
	8	4	100	0	127
Mould mites	Control	4	0	0	2841
	0.5	1	83.6	-	1380
	1	4	93.1	3.11	2841
	1.5	3	97.7	1.43	1461
	2	4	98.5	1.06	2841
	3	3	99.1	0.87	1461
	4	4	99.5	0.45	2841
	8	4	100	0	2841

Pest, life stage	Duration	Replicates	Mean % mortality	SEM	n1
	16	1	100	-	1380
White footed house ant	Control	4	5.2	2.04	359
	0.5	1	100	-	29
	1	4	100	0	329
	1.5	3	100	0	289
	2	4	100	0	301
	3	3	100	0	290
	4	4	100	0	295
	8	4	100	0	330
	16	1	100	-	30
Nurseryweb spiderlings	Control	4	4.4	1.50	205
	0.5	1	100	-	34
	1	4	100	0	189
	1.5	3	100	0	154
	2	4	100	0	204
	3	3	100	0	159
	4	4	100	0	193
	8	4	100	0	190
	16	1	100	-	42
American cockroach immatures	Control	3	0	0	94
	1	3	91.0	4.74	106
	1.5	3	96.1	3.92	98
	2	3	99.1	0.90	98
	3	3	100	0	92
	4	3	100	0	93
	8	3	100	0	97
American cockroach adults	Control	3	4.8	4.76	22
	1	3	87.8	6.19	17
	1.5	3	95.2	4.76	19
	2	3	100	0	20
	3	3	100	0	20
	4	3	100	0	16
	8	3	100	0	23
False katipo spiderlings	Control	4	4.2	2.95	81
	0.5	1	100	-	24
	1	4	100	0	94
	1.5	3	100	0	69
	2	4	100	0	90
	3	3	100	0	67
	4	4	100	0	90
	8	4	100	0	85
	16		100	0	27
Greedy scale	Control	4	43.0	8.55	1049
	0.5	1	100	-	544
	1	4	100	0	737
	1.5	3	100	0	229
	2	4	100	0	680
	3	3	100	0	272
	4	4	100	0	719
	8	4	100	0	708
	16	1	100	-	399

[1] sample size across all replicates

The mortality responses of the most tolerant species are shown in Figure 4 and lethal time estimates for 99.9 percent mortality $LT_{99.9}$ of those test organisms for which the data were appropriate for calculation are shown in Table 3.

Snails were the most tolerant pests and with an estimated exposure time of 11.3 h at 10,000 ppm ozone to obtain 99.9 percent mortality (Figure 4, Table 3); consequently, these treatment parameters were chosen for testing ozone effects on materials described in section 2.

Mould mites, lightbrown apple moth eggs and lightbrown apple moth pupae were the next most tolerant pests, requiring an estimated 2.9-4.3 hours of exposure to 10,000 ppm for 99.9 percent mortality (Table 3). Although lightbrown apple moth pupae were recorded alive 2-3 days after treatment for up to 3 hours of exposure to 10,000 ppm, none of the treated pupae gave rise to adults 8-10 days after a 0.5-hour (1 replicate) or 1-hour (4 replicates) exposure to 10,000 ppm (Table 2).

Exposure of 2-3 hours to 10,000 resulted in complete kill of cockroach immatures and adults (Table 2); however' observations of eggs hatch showed no visual difference in numbers of eggs hatching between the untreated and treated egg cases.

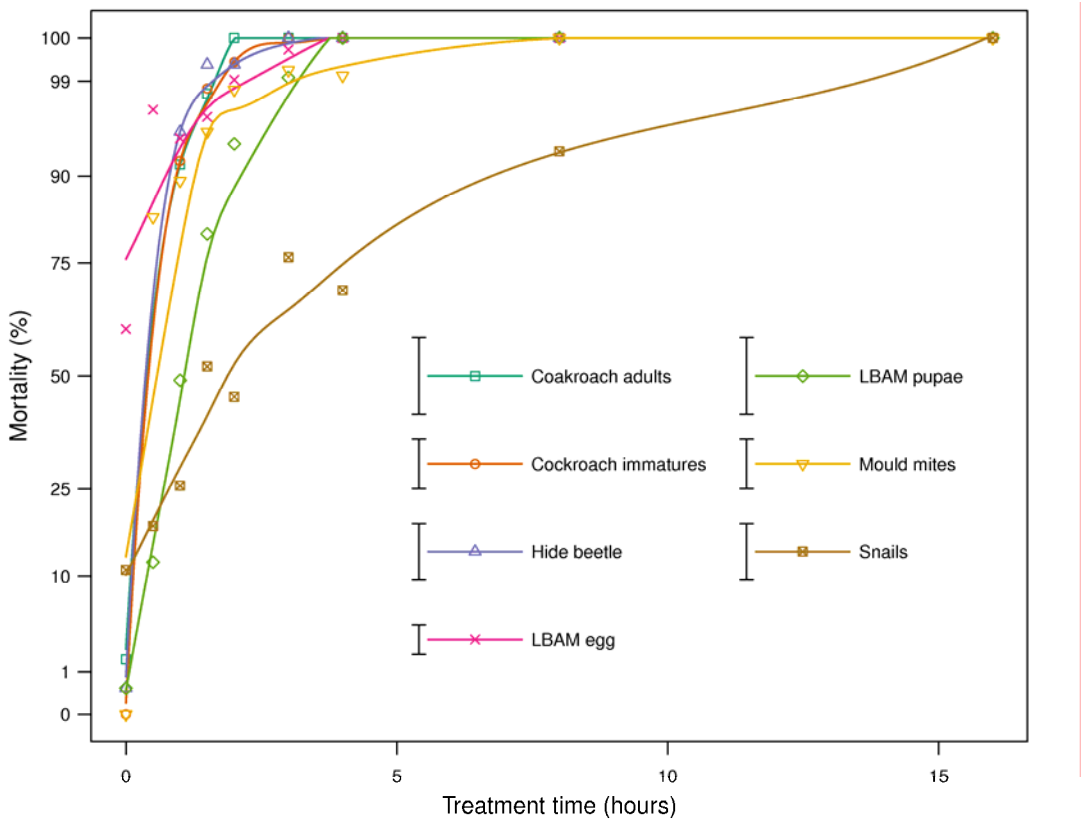


Figure 4: The predicted time (hours) mortality response of cockroach adults and immatures; hide beetle larvae (large); lightbrown apple moth eggs and pupae (assessed 2 days after treatment); mould mites (mixed life stages) and snails (>12 mm) to 10,000 ppm ozone. Points are actual mean mortalities for each time point

Table 3: The estimated lethal times (hours) of exposure to 10,000 ppm ozone for 99.9 percent mortality ($LT_{99.9}$) of the pests where data allowed this prediction

			Replicate			
Pest, life stage	Mean	95% CI	1	2	3	4
Lightbrown apple moth eggs	3.1	2.4-4.2	2.5	3.8	2.4	4.2
Lightbrown apple moth pupae 2 days after treatment	2.9	2.2-3.8	2.9	3.8	2.9	2.2
Snails	11.3	8.6-15.0	10.1	13.4	10.1	12.0
Mould mites	4.3	3.2-5.6	3	6.6	3.6	4.6

4.1.2 Response to dose of application of a 24-hour ozone treatment at 7°C

The fruit pests lightbrown apple moth (eggs and larvae), and greedy scale (immatures and adults) were exposed to 0, 50, 100, 500, 1000 or 2000 ppm for 24 h at 7°C in three experiments as an indication of the effect of lower doses on a Lepidopteran and Hemipteran species for a preliminary investigation in to the feasibility of ozone as a biosecurity and market access treatment . Results are presented below.

4.1.2.1. Ozone concentration and temperature (Experiments 5-7)

General conditions for each experiment and each chamber (ozone dose) are summarised in Table 4. Full ozone and temperature profiles for each fumigation run (i.e. experiment) are shown in Appendices 4 & 5. Ozone doses in chambers targeting 50 ppm averaged 70-75 ppm; targeting 100 ppm averaged 117-129 ppm; targeting 500 ppm averaged 500-520 ppm; targeting 1000 ppm averaged 989-1019 ppm; and targeting 2000 ppm averaged 1765-2006 ppm. Temperature for all experiments averaged 6.5-7.2°C.

Table 4: Desired and actual maximum and minimum mean ozone concentrations and temperatures for chambers and fumigations.

Date	Expt		Chamber	1	2	3	4	5
3/06/2009	5	Ozone	Target (ppm)	50	100	500	1000	2000
			Ramp up[1] (min)	3	6	10	22	79
			Mean ± SEM (ppm)	69.9 ± 0.5	117 ± 0.5	502 ± 10	989 ± 1	1765 ± 4
			Min (ppm)	25	59	222	705	1591
			Max (ppm)	169	238	566	1058	2198
		Temp.	Target (°C)	7	7	7	7	7
			Mean ± SEM (°C)	NR	7.08 ± 0.02	7.21 ± 0.01	7.08 ± 0.02	6.93 ± 0.01
			Min (°C)	NR	6.14	6.29	6.54	6.69
			Max (°C)	NR	7.39	7.55	7.54	7.25
4/06/2009	6	Ozone	Target (ppm)	50	100	500	1000	2000
			Ramp up[1] (min)	3	2	7	12	16
			Mean ± SEM (ppm)	75.4 ± 0.5	129 ± 0.5	520 ± 0.5	1019 ± 0.7	2006 ± 0.6
			Min (ppm)	36	95	365	855	1905
			Max (ppm)	131	214	590	1131	2076
		Temp.	Target (°C)	7	7	7	7	7
			Mean ± SEM (°C)	7.1 ± 0.01	6.74 ± 0.02	6.46 ± 0.02	6.72 ± 0.02	6.61 ± 0.01
			Min (°C)	6.61	6.33	5.94	6.27	6.27
			Max (°C)	7.49	7.17	6.95	7.16	6.96
5/06/2009	7	Ozone	Target (ppm)	50	100	500	1000	2000
			Ramp up[1] (min)	3	4	11	17	30
			Mean ± SEM (ppm)	70.0 ± 0.7	119 ± 0.5	500 ± 0.8	992 ± 1	1906 ± 3
			Min (ppm)	20	52	204	734	1420
			Max (ppm)	328	205	543	1092	2133
		Temp.	Target (°C)	7	7	7	7	7
			Mean ± SEM (°C)	7.03 ± 0.01	6.98 ± 0.00	6.89 ± 0.00	6.83 ± 0.01	7.04 ± 0.01
			Min (°C)	6.29	6.8	6.55	6.44	6.57
			Max (°C)	7.22	7.19	7.1	7.11	7.35

[1] The time it took to establish concentration within 5 percent of target.
NR = Not recorded

4.1.2.2. Efficacy against pests

The mean percentage mortalities and total number of pests tested of lightbrown apple moth eggs and pupae and greedy scale insects (mixed life stages) on potatoes after exposure to ozone at doses between 0 and 2000 ppm at 7°C are shown in Table 5.
After 24 h exposure to 70-75 ppm ozone, all greedy scale on potatoes were killed. Lightbrown apple moth eggs were controlled by a 500-520 ppm treatment for 24 hours. A single lightbrown apple moth pupa was still moving 2-3 days after a 24-hour exposure to 989-1019 ppm ozone; however, no adults emerged after treatment with 500-520 ppm for 24 hours.

Table 5: Mean percent mortalities and number of pests tested at 0, 50, 100, 500, 1000 or 2000 ppm ozone for 24 hours

Pest, life stage	Target conc. (ppm)	Duration	Replicates	Mean % mortality	SEM	Total pests
Lightbrown apple moth eggs	Control	24	4	60.8	4.18	1915
	50	24	4	72.6	2.67	1807
	100	24	4	77.0	2.30	1634
	500	24	4	100	0	1364
	1000	24	4	100	0	1247
	2000	24	4	100	0	1286
Lightbrown apple moth pupae mortality 2-3 days after treatment	Control	24	4	1.0	0.71	401
	50	24	4	19.3	5.17	393
	100	24	4	74.2	9.92	399
	500	24	4	100	0	399
	1000	24	4	99.8	0.25	401
	2000	24	4	100	0	401
Lightbrown apple moth adult emergence from treated pupae 8-10 days after treatment	Control	24	4	6.00	2.25	401
	50	24	4	98.0	1.70	401
	100	24	4	99.8	0.25	399
	500	24	4	100	0	399
	1000	24	4	100	0	399
	2000	24	4	100	0	401
Greedy scale	Control	24	3	15.7	2.43	1177
	50	24	3	100	0	1384
	100	24	3	100	0	1776
	500	24	3	100	0	1631
	1000	24	3	100	0	619
	2000	24	3	100	0	611

The mortality responses of lightbrown apple moth eggs and pupae (2 d and 8-10 d after treatment) are shown in Figure 5, and lethal time estimates for 99.9 percent mortality ($LT_{99.9}$) of lightbrown apple moth eggs and pupae 2-3 days after treatment are shown in Table 6.

A 24-h exposure to 1091 ppm ozone would be required to kill 99.9 percent of lightbrown apple moth eggs. This prediction was longer than that calculated for 99.9 percent mortality of lightbrown apple moth pupae 2-3 days after treatment because the mortality response line was much flatter. It was estimated that a dose of 274 ppm ozone for 24 hours was required to render 99.9 percent of lightbrown apple moth pupae dead if assessed 2-3 days after treatment.

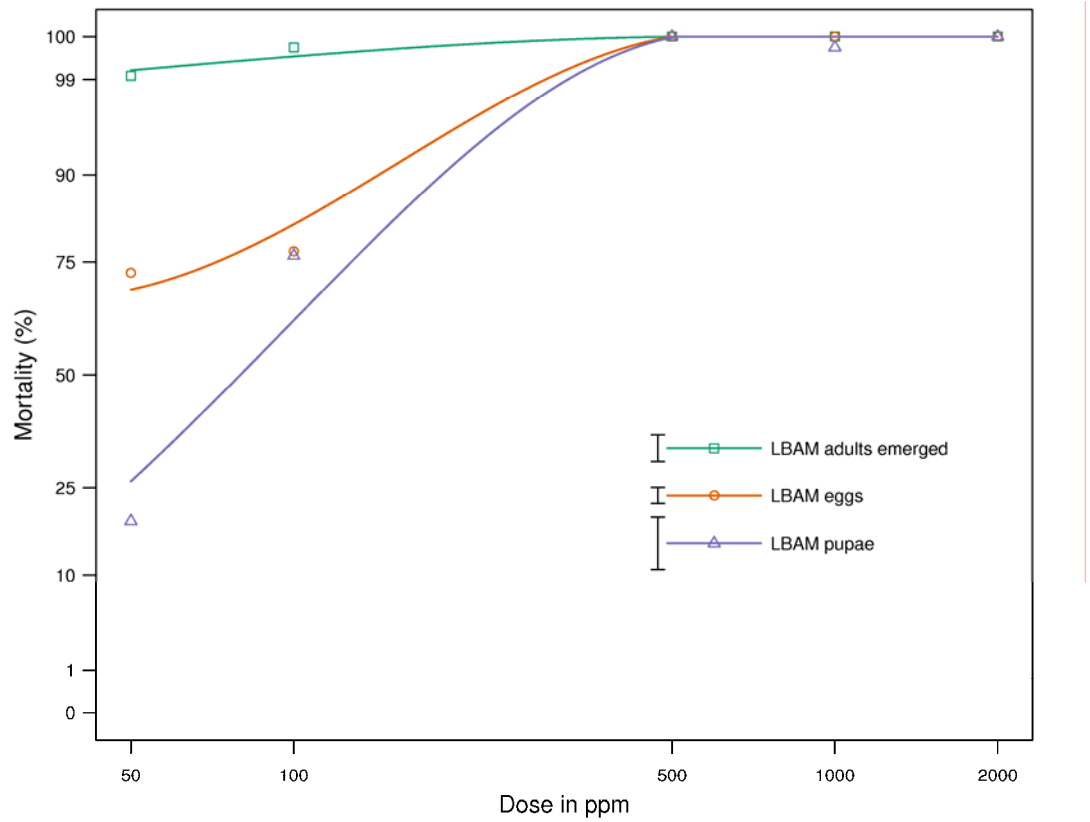


Figure 5: The predicted ozone dose (ppm) mortality response of lightbrown apple moth eggs and pupae assessed 2-3 days after treatment and 8-10 days after treatment after adult emergence in controls, after exposure to ozone for 24 hours at 7°C. Points are actual mean mortalities for each dose point.

Table 6: The estimated lethal dose (ppm) of exposure to a 24 hour ozone treatment for 99.9 percent mortality ($LT_{99.9}$) of the pests where data allowed this prediction.

			Replicate			
Pest, life stage	Mean	95% CI	1	2	3	4
Lightbrown apple moth eggs	1091	551-2160	794	1205	1458	1014
Lightbrown apple moth pupae 2 days after treatment	274	138-543	703	178	344	131

4.2. IMPACT OF HIGH CONCENTRATIONS OF OZONE ON THE INTEGRITY OF SELECTED MATERIALS

4.2.1 General Observations

Ozone concentrations in the three treatment vessels were relatively uniform for all three treatments once the concentration had built up to 10,000 ppm (Figure 7), and treatments proceeded without any difficulties.

Plastic sample 2 (PE-HD 20µm) disintegrated during the treatments, and could not be tested. Sample 8 (Natural rubber) had a sticky feel after the treatment. The initial seals on the fumigation chamber (neoprene rubber) cracked after the first arthropod response run (Figure 8). These were replaced with nitrile rubber seals, which also cracked (Figure 8). Silicon seals were subsequently used successfully without ozone damage sustained to them.

Figure 8: Chamber seals damaged by ozone treatment (left = neoprene rubber, right = nitrile rubber).

Some of the inks on corrugated cardboard samples 12 and 13 bleached during treatment. The royal blue printing on the storage box changed to a green colouration (Figure 9). The yellow colour on the plastic-coated multicoloured photocopying paper boxes lightened slightly, although a small natural colour variation was noted in different boxes of this product before ozone exposure (Figure 10). The red and black inks on the latter boxes were not affected. In addition, insect containers marked with a permanent EXT waterproof marker used in section 1 of this report were significantly faded, while those marked with Stephens Vivid permanent waterproof marker remained readable.

A visual comparison between the exposed and unexposed container metal samples revealed there were no clear differences between the two. This is discussed further below.

Apart from these samples, none of the other samples showed any outward changes in appearance from the ozone treatment, except for plastic sample 2, high-density polyethylene, which had disintegrated during treatment.

Figure 9: Bleaching of printing inks on ozone-exposed cardboard boxing.

Figure 10: Bleaching of printing inks on ozone-exposed plastic-coated printed cardboard boxing relative to unexposed samples.

It was clear from this study that, after exposure to 10,000 ppm ozone for about 11 hours, some printing inks on cardboard packaging could be affected, with colour or shade (typically lightening) changes possible. This is not unexpected, as ozone can readily react with unsaturated bonds present in chromophores (chemical groups giving rise to colour) in organic pigments and dyestuffs, and which will normally reduce the intensity of their colour and/or result in shade changes.

4.2.2 Tensile Testing

Summary of results from the tensile testing, burst strength testing and general and microscopic observations are presented in Table 9. Full details of the results are summarised in Appendix 6. A description of the calculation of tensile properties is described in Appendix 7.

4.2.2.1. Tensile Properties of Plastic Materials

The data from this study suggested that exposure to ozone of films of plastics of type 1 (PET), type 6 (PS), and one example of type 7, namely nylon, results in no reduction in tensile strength and extensibility of these materials. In contrast, for films of plastic type 2 (PE-HD), type 3 (PVC), type 4 (PE-LD), and type 5 (PP), ozone exposure appears to reduce the tensile strength and extensibility of the materials significantly, and in the case of PE-HD rather dramatically so. In addition, films of PE-LD, PP, and PVC exposed to ozone showed increases in film thicknesses relative to the unexposed samples, in some cases by as much as 50 percent (e.g., PP). It needs to be noted that the plastics type 7 category covers a number of plastics not covered by the other categories and only one of them (nylon) was tested in this work. Some other plastics in this category could also be negatively affected by exposure to ozone.

Many plastics are anisotropic, which means that they exhibit different physical properties in different directions. For example, sheets of plastic made as continuously extruded film, which are then stretched or annealed during the manufacturing process, may have different properties (such as break factor, percent elongation at break or tensile strength) along the length of the film or at right angles to the direction of manufacture. This is illustrated, for example, in the tensile properties of the PP film used in this study, where significant differences in tensile strength and percent elongation at break were observed for unexposed films in different directions (Table 7). After exposure to ozone, however, some of these differences were less significant and in some cases there were no longer differences, e.g.

percent elongation at break was the same in either direction, reflecting changes to the anisotropy as a result of the ozone treatment (Table 8). For the PP film, ozone treatment resulted in a highly significant decrease in percent elongation at break compared with the untreated film with the lower values, meaning the films were less extensible or perhaps could be considered as 'brittle' (Table 9). These results reflect the fact that ozone clearly reacted with the PP film, but for other films it does not and no changes occur (e.g., see the data in Table 10 for PET before and after ozone treatment).

There was often large variation for many of the parameters determined between the five subsamples tested for each of the three replicate samples, which was reflected in the large standard deviations often observed. Such variation is very typical for plastic films, where a large variety of factors dictate film strengths and properties, hence the need for statistical analysis to look at the significance of changes after ozone treatment. There are no large errors introduced by other factors.

Tensile strength and tensile strength at break were often the same for many samples (e.g., see the data in Table 3 and others). This was simply a reflection of the fact that the maximum load was the same as the load at break.

The properties of the plastic films vary from one type of plastic to another, both before and after ozone exposure, and there is no one single measure that captures all the physical properties of the materials. Hence a range of tensile parameters are normally reported when such properties are measured, such as those defined by the various test methods. If one were asked to identify one key parameter, that would probably be tensile strength; however, depending on the end use for a material, other properties such as percent elongation at break may be more critical to their performance during use.

The plastic films treated in this study had thicknesses typically used in packaging. However, as these may vary in thickness depending on their duty or role, impacts from treatments may differ depending on film types and thicknesses. In addition, both sides of the packaging film were exposed to ozone in this work. This could mean that the current results present worst case scenarios, as outcomes could be different if only one side of the packaging films were exposed. This might occur in situations where only the exterior side of packaging or wrapped or packaged products were exposed to ozone, and penetration was limited.

Most packaging films that are printed are treated by corona discharge, which generates low levels of ozone at the film surface, to prepare the surfaces so that printing inks will adhere and remain fast. However, the concentrations and times used in the current treatments manifestly exceed those used in print-prepare processes, so any structural damage to films from the current treatments is likely to be much more obvious and extensive. Presumably, similar damage will occur to other products made from plastics examined in this study, but the extent to which it affects the products may vary. Factors such as whether the reaction is confined just to material surfaces or can readily proceed throughout the material, and the physical dimensions of the products, will no doubt play a role. For example, it was observed that one polyliner commonly used for lining kiwifruit cartons disintegrated when exposed to 2000 ppm ozone (it was made of PE-HD). This suggests that this material is very susceptible to ozone damage that is not just confined to its surface, as the structural integrity of the whole product seemed to be affected.

4.2.2.2. Tensile Properties of Rubber Materials

The stickiness noted on the natural rubber after ozone treatment was not unexpected, as this is often observed at the surface of this type of rubber after ozone attack. The natural rubber also

did not perform as well as the synthetic EPDM rubber, but this is also expected. EPDM rubber is especially formulated to be ozone resistant and is typically used in rubber seals for containers, according to Container Repair Services [1, 6].

4.2.3 Burst Strength of Corrugated Cardboard Samples

The results from this study would suggest that lighter-weight cardboard packaging might suffer some reduction in physical properties as a result of high-level ozone treatment. However, interpretation of the results from the current study does need to be made with caution. For example, the boxing was treated with ozone on both sides in the current evaluation but, for some packaged products in a container disinfestation, this might not always be the case. The glue used to construct cardboard boxes was not formally tested in this trial; however, it was noted that some entire boxes treated with high concentrations of ozone fell apart after transportation from Palmerston North to Auckland.

4.2.4 Container Metal Analysis

Based on the results of the comparative visual examination and the tape-type adhesion tests (where applicable) carried out on painted and unpainted metal panels exposed to ozone, the following conclusions can be made:

- Visually there was no difference between ozone-exposed and unexposed control panels, apart from a small change in colour of the C1 painted panel.
- Under the microscope, there were a number of features detected in the painted panels such as raised features, textured surfaces and cracks present around corroded regions. These characteristics were present in both the ozone-exposed specimens and unexposed control specimens and were therefore not considered to be a result of ozone exposure.
- On examination by microscope, there were no significant effects observed, resulting from exposure to ozone on either the painted or the unpainted metal panels, with the exception that the ozone-exposed specimen of sample C2 had some unidirectional cracks on it. These cracks were associated with an area of deformation on this specimen and therefore may not be a result of exposure to ozone.

Results from the crosshatch paint adhesion test as classified in the standard are summarised in Table 10.

Overall, it would appear that ozone had little, if any, effect on the visual appearance and adhesion properties of the paint (as measured by the tape test) on the three painted metal shipping container sections.

Table 9: Summary of measured responses in ozone-treated materials compared with untreated materials.

Measure	PET	PE-HD	PVC	PE-LD	PP	PS	Nylon	Natural Rubber	EPDH Rubber	Cardboard 12	Cardboard 13
Energy to break, MJ/m^3	↓	×	↓	↓	=	=	=				
Break factor, kN/m	=	×	=	↓	=	=	=				
Tensile strength, MPa	=	×	↓	↓	↓	↓	=	=	=		
Tensile strength at break, MPa	=	×	↓	↓	↓	↓	=				
Percent elongation at break, %	=	×	↓	↓	=	=	=	↓	=		
Thickness	↓	×	↑	↑	↑	=	↓				
Burst strength										=	↓
	C1	C2	C3	UC1	UC2	UC3					
Colour change	Yes	No	No	No	No	No					
Cracks/blisters	No	No	No	No	No	No					

↓ indicates lower compared with untreated, = indicates not significantly different from untreated, ↑ indicated higher than untreated.
× indicates sample destroyed by ozone treatment
PET = Polyethylene terephthalate, PE-HD = High-density polyethylene, PVC = Polyvinylchloride, PE-LD = Low-density polyethylene, PP = Polypropylene, PS = Polystyrene, EPDH Rubber = Ethylene Propylene Diene Rubber.

Table 10: Results of the crosshatch and X cut tape tests on ozone-treated materials compared with untreated materials.

Sample ID	Grade allocated	
	Non exposed control	Ozone exposed
C1	5A, [a]	5A, [a]
C2	4A, 3B	4A, 2B
C3	4A, 3B	4A, 3B

[a] The cross hatch tape test was not completed on this specimen, see text.

Note:

1) 'A' grades refer to the X cut tape test; 'B' grades refer to the crosshatch tape test.
2) Grades are allocated between the two extremes of 5 and 0.
3) The descriptions for each grade as provided in ASTM D3359 are as follows:

5A No peeling or removal
4A Trace peeling or removal along incisions or at their intersection
3A Jagged removal along incisions up to 1.6 mm on either side
2A Jagged removal along most of incisions up to 3.2 mm on either side
1A Removal from most of the area of the X under the tape
0A Removal beyond the area of the X

5B The edges of the cuts are completely smooth. None of the squares of the lattice is detached
4B Small flakes of the coating are detached at intersections, less than 5 percent of the area is affected
3B Small flakes of the coating are detached along edges and at intersections of cuts. The area affected is 5 to 15 percent of the lattice
2B The coating has flaked along the edges and on parts of the squares. The area affected is 15 to 35 percent of the lattice
1B The coating has flaked along the edges of cuts in large ribbons and whole squares have detached. The area affected is 35 to 65 percent of the lattice
0B Flaking and detachment worse than Grade 1.

5. Discussion

For the purpose of treating incoming shipping containers, the requirements from MAF-BNZ for treatment include: (1) the treatment duration <24 hours and preferably a few hours; (2) the treatment to be effective against a wide range of pests. The use of ozone as a fumigant against arthropods has primarily been developed for control of pests of stored products (Mason et al. 1999; Leesch & Tebbets 2002; Frazer 2004). The storage times enables the use of long treatments at low concentrations ranging from 5-120 ppm (Manson et al. 1997; Kells et al. 2001; Strait et al. 1998).

Pest species vary in their mortality responses to ozone, for example a 70 minute 600 ppm ozone treatment caused 100 percent mortality of sawtoothed grain beetle (*Oryzaephilus surinamensis*), while 100 hours was required at the same concentration to kill rice weevil (*Sitophilus oryzae*) (Yoshida 1975). This study also has highlighted a large range in the mortality response of invertebrate pests from different families to ozone treatment.

This is the first report on the efficacy of ozone against snails, which was the most tolerant pest tested in this trial, requiring 11.4 hours of exposure to 10,000 ppm ozone. Mould mites, lightbrown apple moth eggs and lightbrown apple moth pupae were the next most tolerant pests, requiring an estimated 2.9-4.3 hours of exposure to 10,000 ppm for 99.9 percent mortality. Although lightbrown apple moth pupae were recorded alive 2-3 days after treatment for up to 3 hours of exposure to 10,000 ppm, none of the treated pupae gave rise to adults 8-10 days after exposure to 10,000 ppm, indicating that those live treated pupae were functionally dead. Using a lower dose for a longer period shows potential for controlling lightbrown apple moth, scale and, most likely, the other non-snail pests. It was estimated that 274 and 1091 ppm over 24 hours was required to kill 99.5 percent of lightbrown apple moth pupae and eggs respectively. Other researchers have shown that exposure to 95-600 ppm for 0.5-6 hours can control 95-100 percent of flour beetles (*Tribolium* sp.) and biting gnats (Erdman 1980; Akey 1982).

Spiders were very susceptible to 10,000 ppm with no survivors after 0.5 or 1 hour. All beetles, ants, cockroaches and scale insects were controlled in 0.5-2 hours when exposed to 10,000 ppm ozone. The eggs of these pests were not formally tested; however, observations indicate that cockroach egg cases are more tolerant than the other life stages. Armstrong (2008) also found that eggs of the coffee berry borer were more tolerant of ozone than the other life stages with eggs surviving a 6-h 10,000 ppm ozone treatment combined with vacuum (-25.4 cm Hg). Dose response experiments showed that 24 hours of exposure to low doses (70-75 ppm) of ozone killed all greedy scale.

Although thrips and mealybugs were not formally investigated in this study, they are most likely among the more susceptible of species, as Hollingsworth & Armstrong (2005) found that more than 98 percent of longtailed mealybugs and adult female western flower thrips were killed by a 1-hour fumigation with ~400 ppm ozone in combination with vacuum in pure CO_2 at 37.8^{o}C, and it was estimated that ~1000 ppm would be sufficient to provide complete mortality.

Tahoe Food Technology of Sparks, Nevada (USA) developed a fumigation technology called Ozofume® that uses ozone under partial vacuum as a treatment against quarantine pests, to prevent gas loss and to enhance target mortality response. A direct comparison of ozone applied at ambient pressure and ozone applied at negative pressure is not available. However, because this combination or partial vacuum and ozone fumigation has been commercialised,

we presume that unpublished proof of enhanced efficacy using ozone when combined with vacuum does exist. Ozone applied under vacuum was investigated for the control of long-tailed mealybugs (*Pseudococcus longispinus*), western flower thrips (*Frankliniella occidentalis*) and coffee berry borer (*Hypothernemus hampei*) (Hollingsworth & Armstrong 2005; Armstrong 2008).

Recent research at the University of California (Lagunas-Solar 2006) and at PFR in New Zealand (Zulhendri et al. in prep.) indicates that using pressure and vacuum cycles combined with a low dose of ethanol vapour has the potential to disinfest mealybugs, leafrollers and scale insects. Pressure/vacuum cycles may enhance the efficacy of ozone against pests (e.g. the most tolerant snail), enabling shorter treatments at ozone concentrations less than 10,000 ppm.

From the materials testing trial and analyses carried out to date it would appear that appropriately specified shipping container materials may not be affected negatively by high-level ozone disinfestation treatments such as those investigated in this work. However, some types of commonly used plastic packaging materials (i.e. high-density polyethylene, PE-HD) ink and rubber could be negatively affected.

Based on overseas findings, ozone had potential to control pests found on surface or in cracks and crevices/tunnels open to surface on dry product. Ozone is probably not going to disinfest cargo sealed in plastic bags/containers. There is potential to investigate lower doses that may be less harsh on materials, such as PE-HD, ink and rubber. Gaining a better understanding of the mortality response of a wide range of resident and unwanted organisms to various doses will enable several ozone treatment protocols to be developed depending on the type of pest intercepted, and the most likely pest/s on a pathway. Options to enhance the uniformity of ozone gas through a container loaded with cargo, includes utilising small pressure/vacuum cycles.

This research has established a proof of concept that fumigation with 10,000 ppm ozone can control a wide range of invertebrate pests. Ozone may have utility inboth biosecurity applications at the border and also for use on exported products to address market access requirements in our key markets.. In the future stakeholders with an interest ineither biosecurity and/or market access will requirea range of disinfestation treatment options, of which ozone may be one, as alternatives to methyl bromide.

6. Acknowledgements

Thanks to Anne Barrington for supply of lightbrown apple moth, Mark Self for supply of nurseryweb spider, Cor Vink for supply of false katipo spiders. Dirk Hasselhoff (Ozone Technologies) for supply and setting up of ozone delivery and monitoring system and Patrick Connolly for statistical analysis of mortality data. We gratefully acknowledge the advice, discussions and/or provision of materials by Martin Pidcock, Aperio Flexipac, Christchurch; Mike Murphy, Elldex Packaging Group, Christchurch; Ben Lazarus, Croxley Stationery Ltd, Auckland; Mike Christmas, Skellerup Industries Ltd, Christchurch; Dr Diana Siew and Clive Stirling IRL Auckland; Len Harvey and Dr Peter Plimmer of the Plastics Centre of Excellence, University of Auckland; and Grant Knight, MAFBNZ, Auckland. Testing was performed by Karla Anderson (container metal samples) and Mike Cooper (plastic, rubber and cardboard) and statistical analysis was by Dr Chikako van Koten. Thanks to Barbara Waddell, Anne Gunson, Garry Hill and Jack Armstrong for editorial comments. The research was funded by MAF BNZ and Foundation for Research Science and Technology, Better Border Biosecurity www.b3nz.org (CO2X0501).

7. References

Achen M, Yousef 2001. Efficacy of ozone against Escherichia coli O157:H7 on apples. Journal of Food Science 66 (9): 1380-1384.

Aguayo E, Escalona VH, Artes F 2006. Effect of cyclic exposure to ozone gas on physicochemical, sensorial and microbial quality of whole and sliced tomatoes. Postharvest Biology and Technology 39(2): 169-177.

Anachkov MP, Rakovsky SK, Stefanova RV, Stoyanov AK 1993. Ozone degradation of polychloroprene rubber in solution. Polymer Degradation and Stability 41(2): 185-190.

Bell CH 2000. Fumigation in the 21st century. Crop Protection 19(8-10): 563-569.

Bialka KL, Demirci A 2007a. Efficacy of aqueous ozone for the decontamination of Escherichia coli O157:H7 and Salmonella on raspberries and strawberries. Journal of Food Protection 70(5): 1088-1092.

Bialka KL, Demirci A 2007b. Utilization of gaseous ozone for the decontamination of Escherichia coli O157:H7 and Salmonella on raspberries and strawberries. Journal of Food Protection 70(5): 1093-1098.

Carlins JJ, Clark RG 1982. Ozone generation by corona discharge. In: Rice RG, Netzer A ed. Handbook of Ozone Technology and Applications. Michigan, Ann Arbor Science. Pp. 1-75.
Cataldo F 2002. On the action of ozone on polyaniline. Polymer Degradation and Stability 75(1): 93-98.

Das E, Gurakan GC, Bayindirli A 2006. Effect of controlled atmosphere storage, modified atmosphere packaging and gaseous ozone treatment on the survival of Salmonella Enteritidis on cherry tomatoes. Food Microbiology 23(5): 430-438.

Davenport TL, Burg SP, White TL 2006. Optimal low-pressure conditions for long-term storage of fresh commodities kill Caribbean fruit fly eggs and larvae. Horttechnology 16(1): 98-104.

Del Agricultural 2000. Ozone solutions for the Agricultural Industry: Complete understanding of ozone use and technology. www.delozone.com/Pages/agozonefact.html

Dentener PR, Bennett KV, Hoy LE, Lewthwaite SE, Lester PJ, Maindonald JH, Connolly PG 1997. Postharvest disinfestation of lightbrown apple moth and longtailed mealybug on persimmons using heat and cold. Postharvest Biology and Technology 12(3): 255-264.

Diao HF, Li XY, Gu JD, Shi HC, Xie ZM 2004. Electron microscopic investigation of the bactericidal action of electrochemical disinfection in comparison with chlorination, ozonation and Fenton reaction. Process Biochemistry 39(11): 1421-1426.

Drake SR, Hansen JD, Elfving DC, Tang J, Wang S 2005. Hot water to control codling moth in sweet cherries: Efficacy and quality. Journal of Food Quality 28(4): 361-376.

Fields P, White D 2002. Alternatives to methyl bromide treatments for stored-product and quarantine insects. Annual Review of Entomology 47: 331-359.

Fuester RW, Kozempel MF, Forster LD, Goldberg N, Casillas LI, Swan KS 2004. A novel nonchemical method for quarantine treatment of fruits: California red scale on citrus. Journal of Economic Entomology 97(6): 1861-1867.

Giles J 2005. Hikes in surface ozone could suffocate crops. Nature 5 May 2005: 7.

Gimeno BS, Bermejo V, Reinert RA, Zheng Y, Barnes JD 1999. Adverse effects of ambient ozone on watermelon yield and physiology at a rural site in Eastern Spain. New Phytologist 144(2): 245-260.

Gullino ML, Clini C, Garibaldi A 2005. Life without methyl bromide: the Italian experience in replacing the fumigant. Communications in Agricultural and Applied Biological Sciences. Ghent Belgium, Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen. Pp. 13-25.

Hill AG, Rice RG 1982. Historical background, properties and applications. In: Rice RG, Netzer A ed. Handbook of Ozone Technology and Applications. Michigan, Ann Arbor Science. Pp. 1-40.

Hollingsworth RG, Armstrong JW 2005. Potential of temperature, controlled atmospheres and ozone fumigation to control thrips and mealybugs on ornamental plants for export. Journal of Economic Entomology 98(2): 289-298.

IPCS IPoCS, CEC CfEC 1993. Ozone. http://www.cdc.gov/niosh/ipcsneng/neng0068.html [accessed 27th October 2008].

Kefeli AA, Razumovskii SD, Zaikov GY 1971. Interaction of polyethylene with ozone. Polymer Science U.S.S.R. 13(4): 904-911.

Kim JG, Yousef AE, Khadre MA 2003. Ozone and its current and future application in the food industry. Advances in Food and Nutrition Research. Pp. 167-218.

Leesch J, Tebbets J 2002. The mortality of Indian meal moth and confused flour beetle after exposure to ozone. Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions.

Macmanus LF, Walzak MJ, McIntyre NS 1999. Study of ultraviolet light and ozone surface modification of polypropylene. Journal of Polymer Science, Part A: Polymer Chemistry 37(14): 2489-2501.

Mason LJ, Woloshuk CP, Maier DE 1997. Efficacy of ozone to control insects, molds and mycotoxins, pp 665-670. In: Donahaye EJ, Varnava A [eds], Proceedings of the International Conference on controlled atmosphere and fumigation in stored products, Nicosia, Cyprus Printer Ltd, Nicosia.

Mason LJ, Woloshuk CP, Maier DE 1999. Controlling stored grain insects with ozone fumigation. In: Stored Product Protection: Proceedings of the 7th International Working Conference on Stored-Product Protection, Beijing, China, 14-19 October, 1998, pp. 536-547.
Mathieson I, Bradley RH 1996. Improved adhesion to polymers by UV/ozone surface oxidation. International Journal of Adhesion and Adhesives 16(1): 29-31.

Nair TM, Kumaran MG, Unnikrishnan G, Kunchandy S 2008. Ageing studies of ethylene propylene diene monomer rubber/styrene butadiene rubber blends: Effects of heat, ozone, gamma radiation, and water. Journal of Applied Polymer Science 107(5): 2923-2929.

Najafi MBH, Khodaparast MHH 2009. Efficacy of ozone to reduce microbial populations in date fruits. Food Control 20: 27-30.

Ozen BF, Floros JD 2001. Effects of emerging food processing techniques on the packaging materials. Trends in Food Science & Technology 12(2): 60-67.

Ozen BF, Mauer LJ, Floros JD 2002. Effects of ozone exposure on the structural, mechanical and barrier properties of select plastic packaging films. Packaging Technology and Science 15(6): 301-311.

Palou L, Crisosto CH, Smilanick JL, Adaskaveg JE, Zoffoli JP 2002. Effects of continuous 0.3 ppm ozone exposure on decay development and physiological responses of peaches and table grapes in cold storage. Postharvest Biology and Technology 24(1): 39-48.

Palou L, Smilanick JL, Crisosto CH, Mansour M, Plaza P 2003. Ozone gas penetration and control of the sporulation of *Penicillium digitatum* and *Penicillium italicum* within commercial packages of oranges during cold storage. Crop Protection 22(9): 1131-1134.

Perez AG, Sanz C, Rios JJ, Olias R, Olias JM 1999. Effects of ozone treatment on postharvest strawberry quality. Journal of Agricultural and Food Chemistry 47: 1652-1656.

Rajendran S 2001. Alternatives to methyl bromide as fumigants for stored food commodities. Pesticide Outlook 12(6): 249-253.

Ren Y, Mahon D 2006. Fumigation trials on the application of ethyl formate to wheat, split faba beans and sorghum in small metal bins. Journal of Stored Products Research 42(3): 277-289.

Ren Y, Mahon D, van Someren Graver J, Head M 2008. Fumigation trial on direct application of liquid carbonyl sulphide to wheat in a 2500 t concrete silo. Journal of Stored Products Research 44(2): 115-125.

Selma MV, Beltran D, Allende A, Chacon-Vera E, Gil MI 2007. Elimination by ozone of *Shigella sonnei* in shredded lettuce and water. Food Microbiology 24: 492-499.

Selma MV, Beltran D, Chacon-Vera E, Gil MI 2006. Effect of Ozone on the inactivation of *Yersinia enterocolitica* and the reduction of natural flora on potatoes. Journal of Food Protection 69: 2357-2363.

Selma MV, Ibanez AM, Allende A, Cantwell M, Suslow T 2008a. Effect of gaseous ozone and hot water on microbial and sensory quality of cantaloupe and potential transference of *Escherichia coli* O157:H7 during cutting. Food Microbiology 25: 162-168.

Selma MV, Ibanez AM, Cantwell M, Suslow T 2008b. Reduction by gaseous ozone of Salmonella and microbial flora associated with fresh-cut cantaloupe. Food Microbiology 25(4): 558-565.

Shanbhag PV, Sirkar KK 1998. Ozone and Oxygen permeation behavior of silicone capillary membranes employed in membrane ozonators. Journal of Applied Polymer Science 69(7): 1263-1273.

Sleeper W, Henry D 2002. Durability test results of construction and process materials exposed to liquid and gas phase ozone. Ozone: Science and Engineering 24(4): 249-260.
Smilanick JL, Margosan DM, Mlikota Gabler F 2002. Impact of ozonated water on the quality and shelf-life of fresh citrus fruit, stone fruit, and table grapes. Ozone: Science and Engineering 24(5): 343-356.

Sousa AH, Faroni LRDA, Guedes RNC, Totola MR, Urruchi WI 2008. Ozone as a management alternative against phosphine-resistant insect pests of stored products. Journal of Stored Products Research 44: 379-385.

Taylor RWD 1994. Methyl bromide--Is there any future for this noteworthy fumigant? Journal of Stored Products Research 30(4): 253-260.

Tzortzakis N, Singleton I, Barnes J 2007. Deployment of low-level ozone-enrichment for the preservation of chilled fresh produce. Postharvest Biology and Technology 43(2): 261-270.
Van Epenhuijsen CW, Hedderley DI, Somerfield KG, Brash DW 2007. Efficacy of ethyl formate and ethyl acetate for the control of onion thrips (Thrips tabaci). New Zealand Journal of Crop and Horticultural Science 35(2): 267-274.

Wei K, Zhou H, Zhou T, Gong J 2007. Comparison of aqueous ozone and chlorine as sanitizers in the food processing industry: Impact on fresh agricultural produce quality. Ozone: Science and Engineering 29(2): 113-120.

Whangchai K, Saengnil K, Uthaibutra J 2006. Effect of ozone in combination with some organic acids on the control of postharvest decay and pericarp browning of longan fruit. Crop Protection 25(8): 821-825.

Wikipedia 2008. Ozone. http://en.wikipedia.org/wiki/Ozone [accessed 28th October 2008].
Wojtowicz JA 2005. Ozone. Kirk-Othmer encyclopedia of chemical technology. New York, Wiley. Pp. 768-822.

Xin N, Ren YL, Forrester RI, Ming X, Mahon D 2008. Toxicity of ethyl formate to adult Sitophilus oryzae (L.), Tribolium castaneum (herbst) and Rhyzopertha dominica (F.). Journal of Stored Products Research 44(3): 241-246.

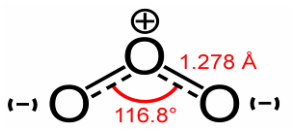
8. Appendices

APPENDIX 1: CHEMICAL AND PHYSICAL PROPERTIES OF OZONE

Ozone (a trioxygen molecule with the chemical designation of O_3) (Figure A1.1a) is a naturally occurring compound, and stratospheric ozone is needed to protect living organisms from negative effects of light in the ultraviolet (UV) wave length. Ozone is formed naturally in the upper atmosphere from oxygen by both UV light and atmospheric electrical discharges (lightning). Ozone is also found in lower levels of the atmosphere where it is produced primarily as a result of photochemical oxidation of hydrocarbons from automobile and industrial emissions, or coincidentally by photocopiers, electrical transformers and other electrical devices (Xiu 1999). Therefore, humans are exposed to low levels of lower atmospheric ozone (about 0.05 ppm at sea level) on a daily basis (Xiu 1999).

General characteristics of ozone include: (1) the ozone molecule is highly unstable and easily degraded to oxygen (O_2) (Figure A1.1b); (2) oxidation at high ozone concentrations forms CO_2 and H_2O (Horvath et al. 1985); (3) ozone has a half-life of 20-30 minutes and leaves no residue once it decomposes to molecular oxygen (Kells et al. 2001); (4) ozone is relatively stable at colder temperatures and the decomposition rate increases as temperature increases (Achen & Yousef 2001); (5) gaseous ozone is more stable than aqueous ozone (Weavers & Wickramanayake 2001); (6) ozone solubility in water is affected directly by pressure and inversely by temperature and it is most soluble at 0°C (= 0.64 L O_3/L water) and becomes insoluble at 60°C and normal atmospheric pressure (Hill & Rice 1982; Wojtowicz 2005); (7) ozone gas is colourless in low concentration and exhibits a blue colour at higher concentrations at normal temperature; (8) ozone has a pungent odour characteristic of electrical sparks that is detectable by the human nose at ≥0.01 ppm; (9) ozone has boiling and melting points of -112°C and -193°C, respectively, and its critical temperature (the temperature at and above which the vapour of the substance cannot be liquefied, no matter how much pressure is applied) is -12°C.

a.



b. $O_3 + M \longleftrightarrow O + O_2 + M$ -106.5 kJ

$O_3 + O \longrightarrow 2O_2$ +391.8 kJ

Figure A1.1: (A). An ozone molecule (Wikipedia 2008); (B) Two steps of decomposition of an ozone molecule and their associated enthalpies. M is any other molecule in the gas mixture (Carlins & Clark 1982).

APPENDIX 2: HEALTH AND SAFETY

Although ozone is a GRAS compound, its oxidative properties can be harmful to human mucosal tissues and, hence, must be treated with caution because ozone is injurious at concentrations > 2 ppm. The limit of occupational exposure for humans is 8 hours/day for 5 days at 0.1 ppm and short-term exposure (15 minutes) at 0.3 ppm. Ozone can be harmful if absorbed through inhalation by affecting the central nervous system and resulting in headaches. Ozone is an irritant that can cause dryness of the mucous membranes of the eyes and nose. However, after the impact of first exposure, subsequent exposures will have lesser effects, suggesting that tolerance to sub-acute ozone exposure develops quickly. Acute exposure at high ozone concentrations can result in lung oedema, which usually does not become evident until several hours after exposure (IPCS & CEC 1993). Exposure to ground level ozone, which is considered a pollutant, is a growing concern because of potential detrimental impacts on both human health and agricultural crops (Gimeno et al. 1999; Giles 2005).

As an oxidant, ozone can react with unsaturated organic compounds to form ozonides that can decompose over time or rapidly in a violent explosion. Ozone also poses a risk of explosion if heated in the presence of certain catalysts, such as hydrogen, copper, iron or chromium. Therefore, care must be taken to avoid contact with all inorganic or organic oxidizable materials. The lower explosion limit for ozone diluted with oxygen at room temperature and normal atmospheric pressure is 10-11 vol%. Ozone at >10-11 vol% can result in an explosive chain decomposition reaction that converts all the ozone to oxygen (Koike et al. 2005).

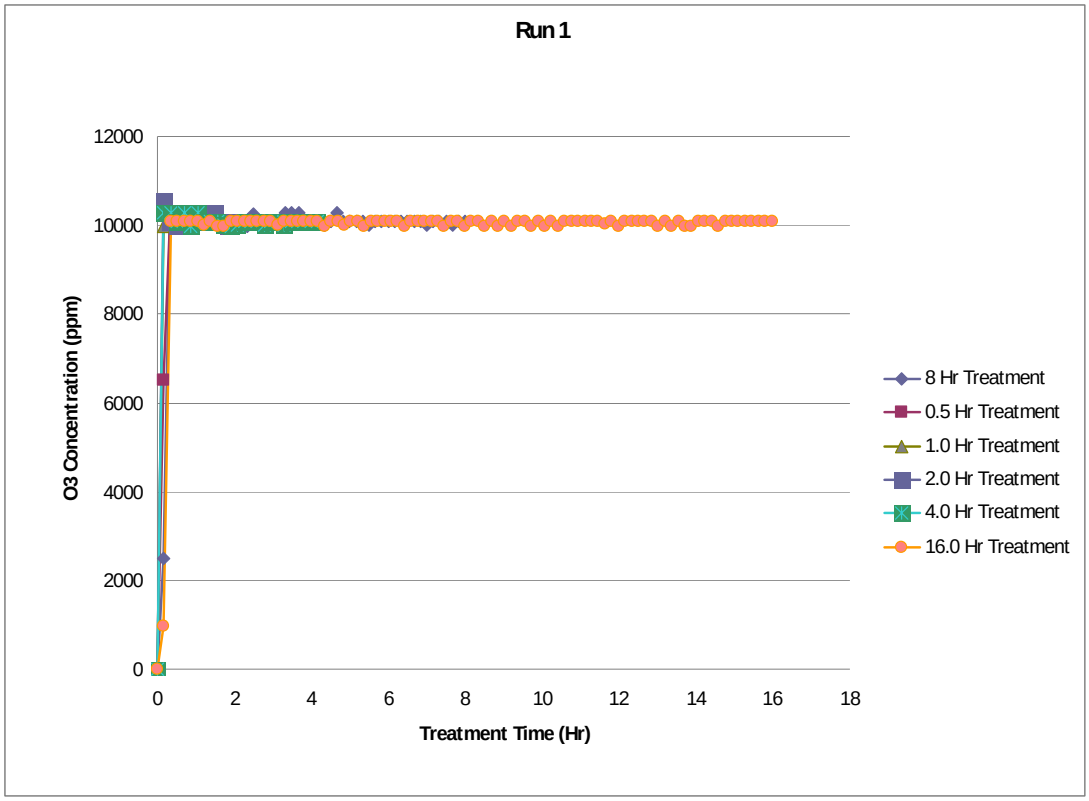
APPENDIX 3: SUMMARY OF LITERATURE REPORTING ON OZONE EFFICACY AGAINST VARIOUS INVERTEBRATES AND PATHOGENS

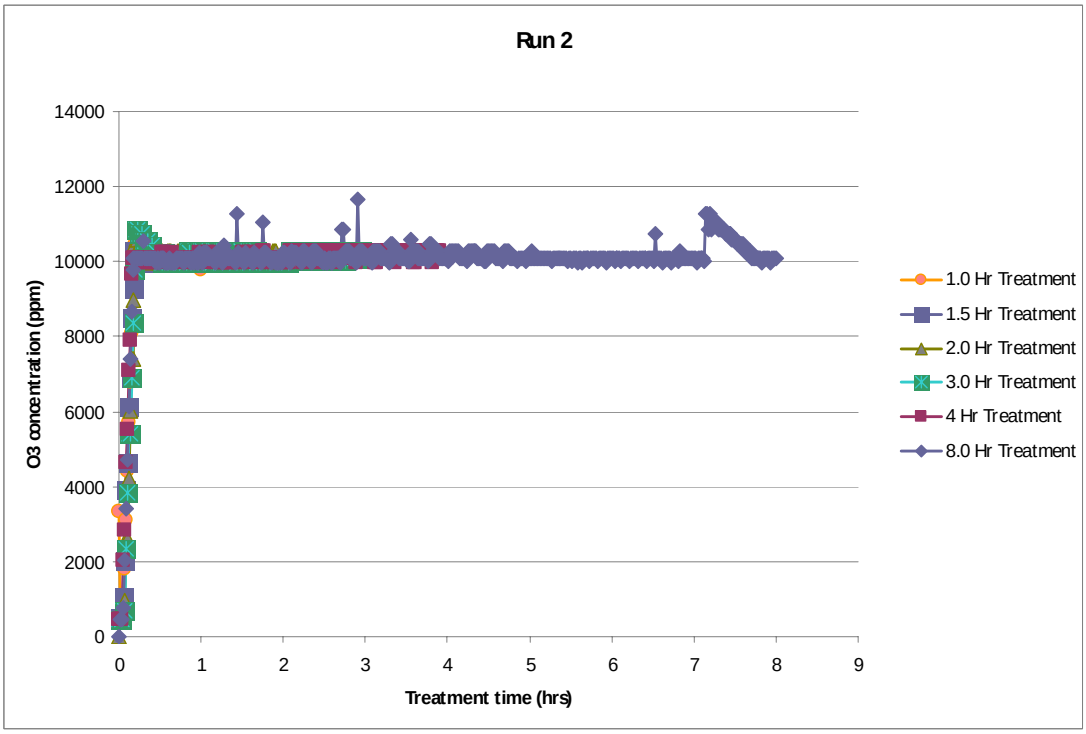
Types	Treatment	Targets	Efficacy*	References**
Gas	0.3 ppm for 22-26 days	Cockroaches (*Periplaneta americana* and *Nauphoela cinerea*) and red imported fire ant (*Solenopsis invicta*)	No effect	Levy et al. (1974)
Gas	5 ppm for 3-5 days	Confused flour beetle (*Tribolium confusum*) and sawtoothed grain beetle (*Oryzaephilus surinamensis*)	100% mortality	Manson et al. (1997; in Kells et al. 2001)
Gas	95-115 ppm ozone for 3.5-6 h	Flour beetles (*Tribolium confusum* and *Tribolium castaneum*)	100% mortality	Erdman (1980)
Gas	50 ppm for 3 days	Confused flour beetle (*Tribolium confusum*), maize weevil (*Sitophilus zeamais*) and Indian meal moth (*Plodia interpunctella*).	100% mortality of flour beetle and maize weevil but not Indian meal moth	Strait (1998; in Kells et al. 2001)
Gas	25-50 ppm for 3-5 days	Red flour beetle (*Tribolium castaneum*), maize weevil (*Sitophilus zeamais*) and Indian meal moth (*Plodia interpunctella*)	>90% except Indian meal moth treated at 25 ppm for 5 days	Kells et al. (2001)
Gas	95-120 ppm	Sawtoothed grain beetle (*Oryzaephilus surinamensis*) rice weevil (*Sitophilus oryzae*)	Grain beetle LT_{95} = 70 min Rice weevil LT_{95} = 100 h	Yoshida (1975)
Gas	600 ppm	Biting gnat (*Culicoides variipennis*)	96% mortality after 30 min 100% mortality after 60 min	Akey (1982)
Gas	Ozone concentrations from 0 to 3800 ppm, treatment durations from 30 to 120 min, vacuums from 0 to 0.41 bar below ambient, temperatures from 32.2-40.6°C and controlled atmospheres	Longtailed mealybug (*Pseudococcus longispinus*) and western flower thrips (*Frankliniella occidentalis*)	Mealybug mortality < 50% after 1 hour treatment 600-1200 ppm ozone applied with vacuum in air. Thrips mortality 97-100% after 1 hour treatment of ~2000 ppm ozone with vacuum in air	Hollingsworth & Armstrong (2005)
Gas	10000 ppm at 13°C and vacuum of -25.4 cm Hg for 6 hours	Coffee berry borer (*Hypothenemus hampei*) and coffee leaf rust (*Hemileia vastatrix*)	Complete mortality of all life stages except eggs (~15% survival)	Armstrong (2008)
Gas	0.1-0.3 ppm for 12 days	Fungal decay on blueberries mainly caused by *Botrytis cinerea*	No fungal decay in treatments, 20% fungal decay in controls	Barth et al. (1995)
Gas	0.35 ppm at 2°C for 3 days	Fungal infection (visual inspection) on strawberries (*Fragaria* x *ananassa* Duch. 'Camarosa')	Poor efficacy	Perez et al. (1999)
Gas	0.3 ppm at 5 °C for 4 weeks	Fungal infection in peach (*Prunus persica* L.): Brown rot (*M. fructicola*) Grey mould (*Botrytis cinerea*) Mucor rot (*Mucor piriformis*) Blue mould (*Penicillium expansum*)	 Good efficacy Poor efficacy Good efficacy Good efficacy	Palou et al. (2002)
Water	10 ppm for 20 minutes	Fungal infection on citrus:		Smilanick et al. (2002)

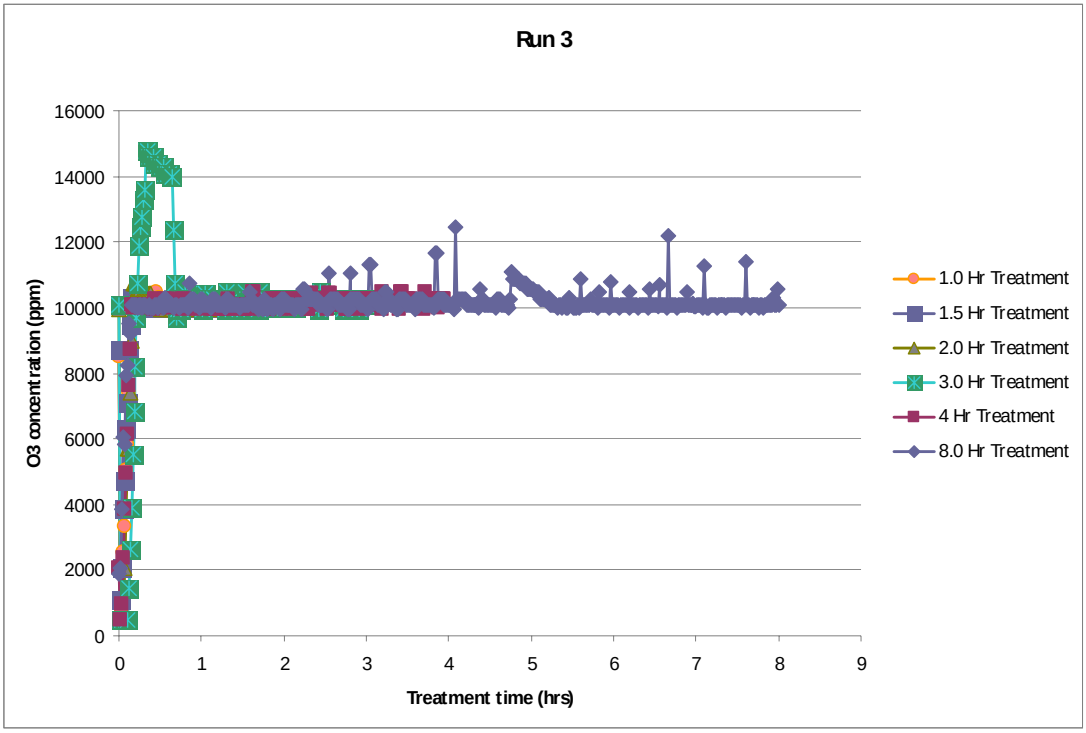
Types	Treatment	Targets	Efficacy*	References**
		Green mould (*P. digitatum*)	Poor efficacy	
		Sour rot (*Geotrichum citri-aurantii*)	Poor efficacy	
	1.5 ppm for 1 minute and 5 ppm for 15 minutes	Fungal infection on peaches: Brown rot (*M. fructicola*)	Good efficacy	
	5 ppm for 1 and 5 minutes	Natural aerobic bacteria, yeasts and filamentous fungal populations	Good efficacy	
Gas	0.72 ppm for 14 days at 12.8°C	Fungal infection on oranges: *P. digitatum* and *P. italicum*	Good efficacy	Palou et al. (2003)
Gas	4 ppm for 30 minutes every 3 h, for 15 days at 5°C	Microbial fungal load on whole and fresh cut tomatoes	Good efficacy	Aguayo et al. (2006)
Gas	10 ppm for 5-20 minutes	*S. enteritidtis* on cherry tomatoes	Good efficacy	Das et al. (2006)
Water	1.4 and 1.9 ppm for 1 minute	*Y. enterolitica* and *L. monocytogenes* on potatoes	Good efficacy	Selma et al. (2006)
Gas	200 ppm for 1-2 hours	Total microorganism population on longan fruit	Good efficacy	Whangchai et al. (2006)
Water	1.7-8.9 ppm at 20°C for 2-64 minutes, 21 ppm at 4°C for 64 minutes *	*Escherichia coli* O157: H7 and *Salmonella*	Good efficacy	Bialka & Demirci (2007a)
Gas	Continuous ozone (5% wt/wt) for 64 minutes followed by pressurized ozone (5% wt/wt, 84 kPa)	*E. coli* O157: H7 and *S. enterica* on raspberries and strawberries	Good efficacy	Bialka & Demirci (2007b)
Water	1.6 and 2.2 ppm for 1 minute, 5 ppm for 5 minutes	*Shigella sonnei* on lettuce	Good efficacy	Selma et al. (2007)
Gas	0.1 ppm for 8 days	*B. cinerea* on Clementine mandarins, tomatoes and plums	Good efficacy	Tzortzakis et al. (2007)
Water	<10 ppm at 4 and 23°C for 3, 5 and 10 minutes*	Total natural flora populations (microbes and fungi) on lettuce and strawberries	Poor efficacy	Wei et al. (2007)
Gas	Combination of 75°C water and 10,000 ppm for 30 minutes	*E. coli* O157: H7 on cantaloupe	Good efficacy	Selma et al. (2008a)
Gas	10,000 ppm for 30 minutes under vacuum	*Salmonella* on fresh-cut cantaloupe	Good efficacy	Selma et al. (2008b)
Gas	1- 5 ppm for 15-60 minutes*	Coliform, *Staphylococcus aureus,* yeasts and moulds on date fruits	Good efficacy	Najafi & Khodaparast (2009)

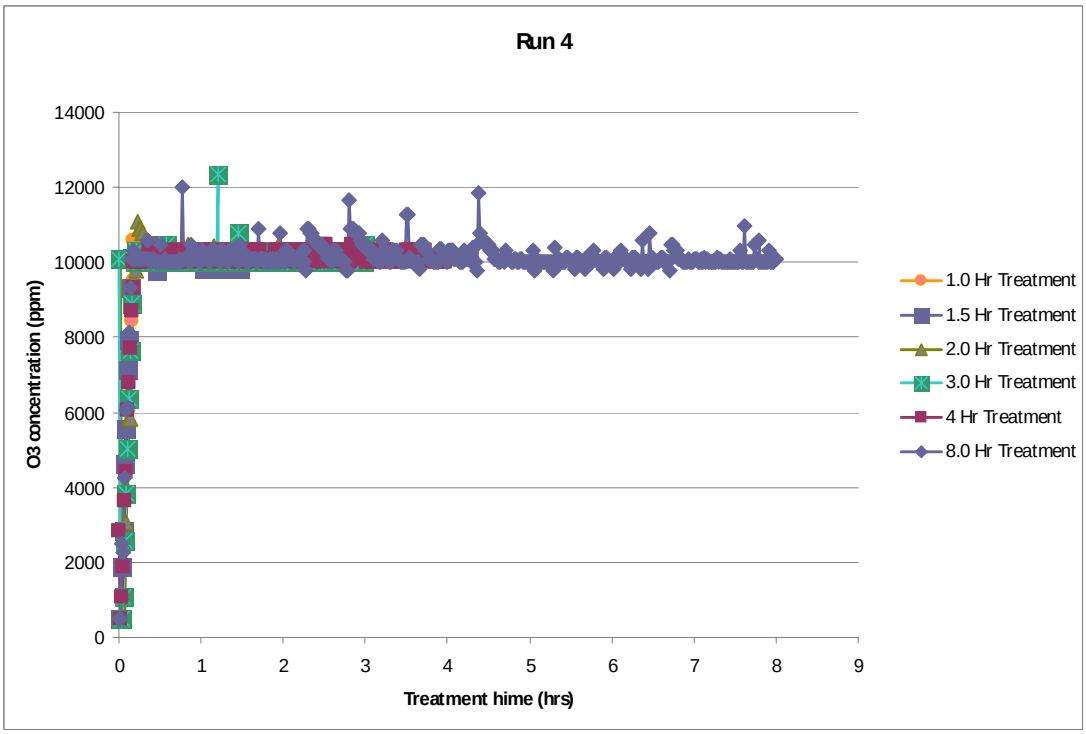
Gas - Gaseous ozone
Water - Ozonated water/ aqueous ozone
* Results vary according to treatments, see article for details
** See Reference section for complete citations

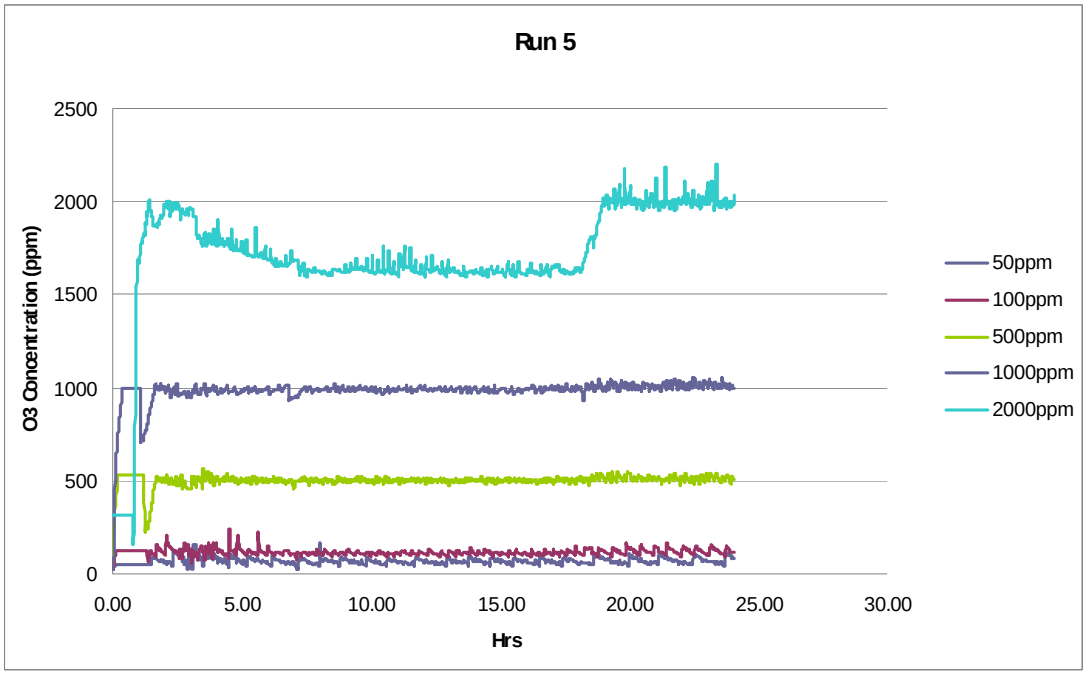
APPENDIX 4: OZONE CONCENTRATION PROFILES FROM EACH OZONE EXPERIMENT

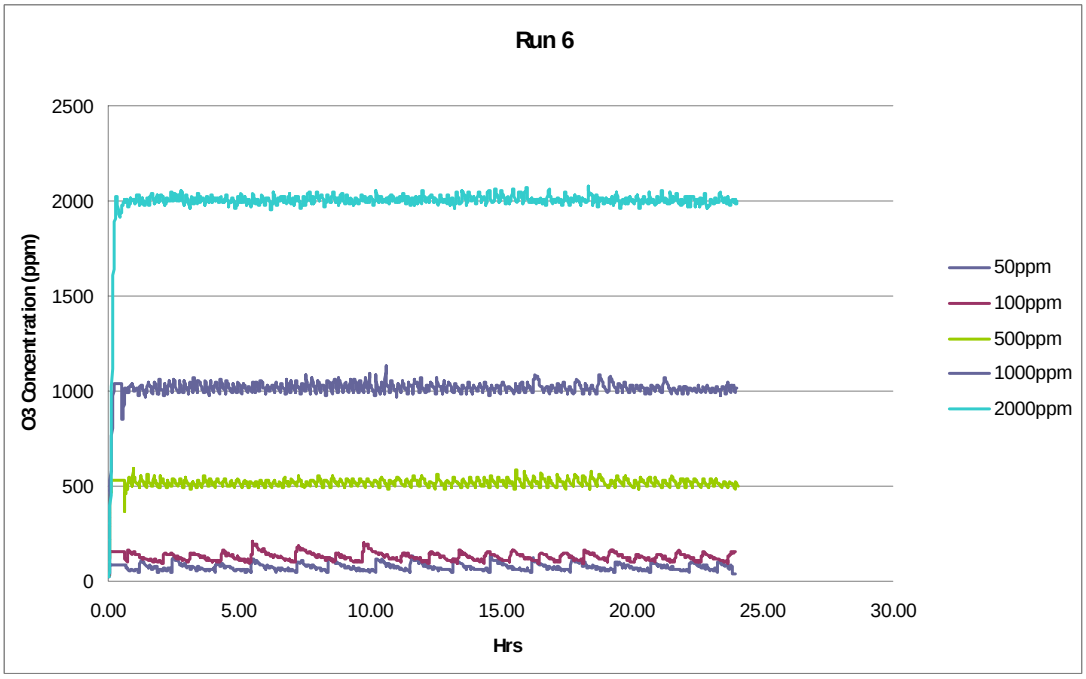


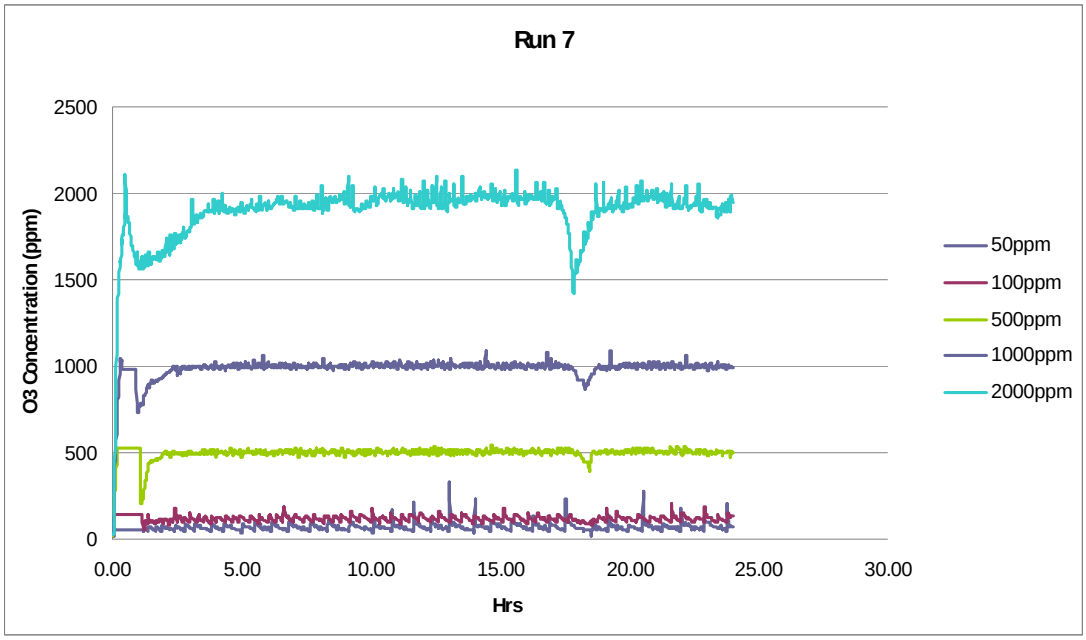



Run 3
16000
14000
12000
10000
8000
6000
4000
2000
0
O3 concentration (ppm)
0
1
2
3
4
5
6
7
8
9
Treatment time (hrs)
1.0 Hr Treatment
1.5 Hr Treatment
2.0 Hr Treatment
3.0 Hr Treatment
4 Hr Treatment
8.0 Hr Treatment

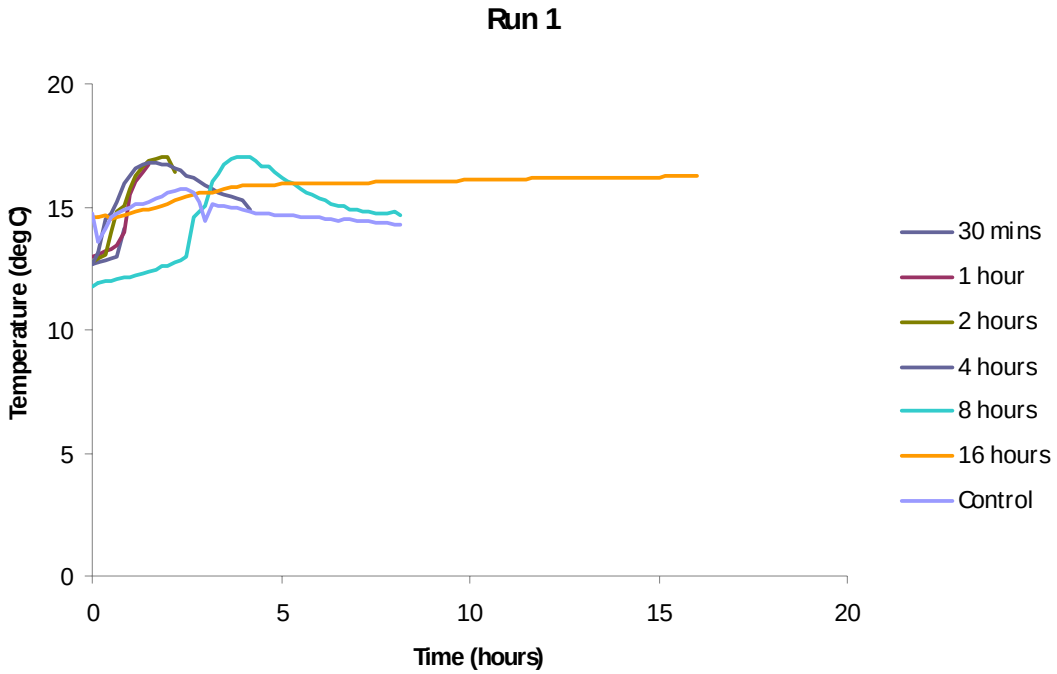

Run 4
14000
12000
10000
8000
6000
4000
2000
0
O3 concentration (ppm)
0
1
2
3
4
5
6
7
8
9
Treatment hime (hrs)
1.0 Hr Treatment
1.5 Hr Treatment
2.0 Hr Treatment
3.0 Hr Treatment
4 Hr Treatment
8.0 Hr Treatment

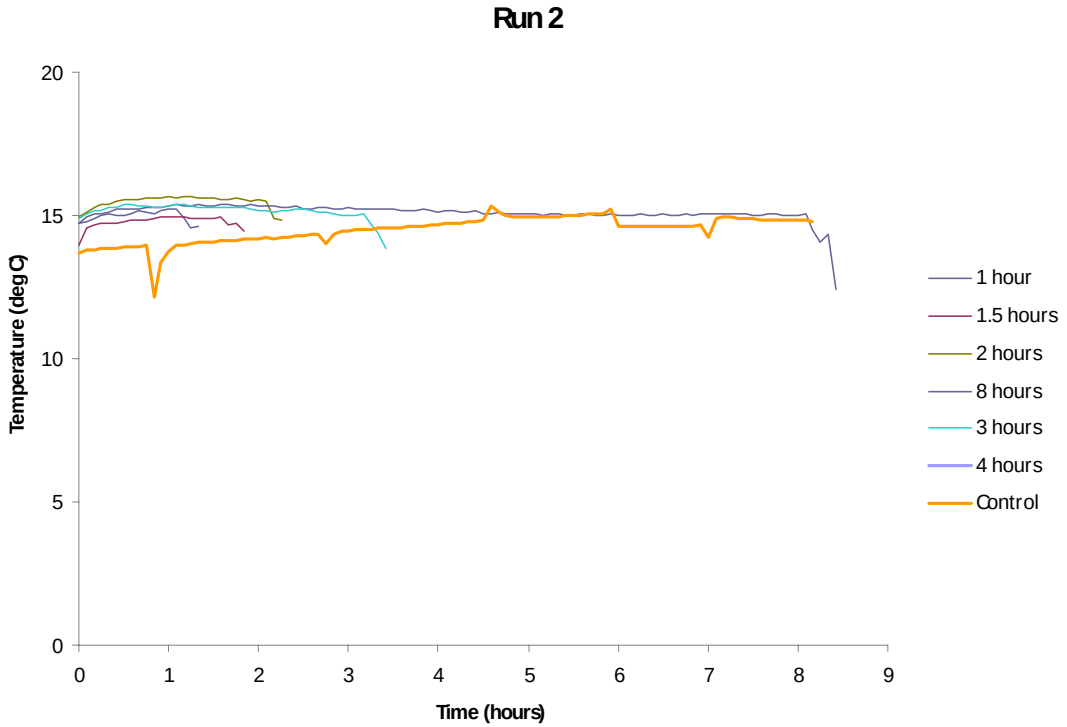

Run 5
2500
2000
1500
1000
500
0
O3 Concentration (ppm)
0.00
5.00
10.00
15.00
20.00
25.00
30.00
Hrs
50ppm
100ppm
500ppm
1000ppm
2000ppm

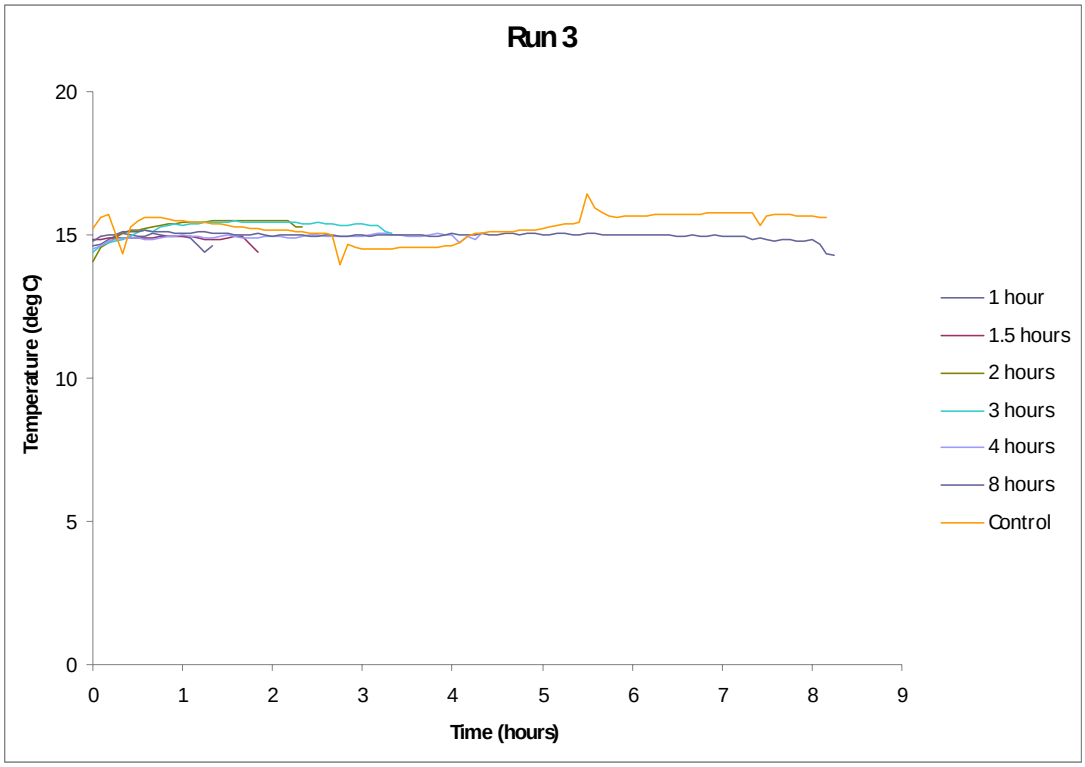

Run 6
2500
2000
1500
1000
500
0
O3 Concentration (ppm)
0.00
5.00
10.00
15.00
20.00
25.00
30.00
Hrs
50ppm
100ppm
500ppm
1000ppm
2000ppm

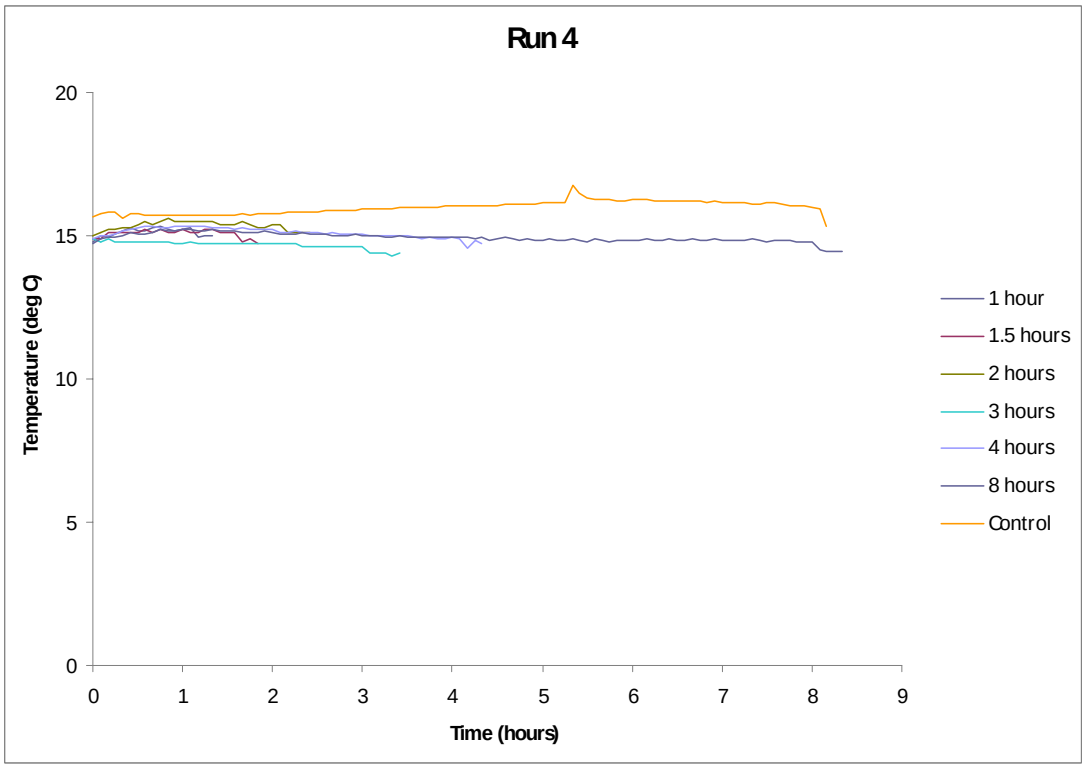

Run 7
2500
2000
1500
1000
500
0
O3 Concentration (ppm)
0.00
5.00
10.00
15.00
20.00
25.00
30.00
Hrs
50ppm
100ppm
500ppm
1000ppm
2000ppm

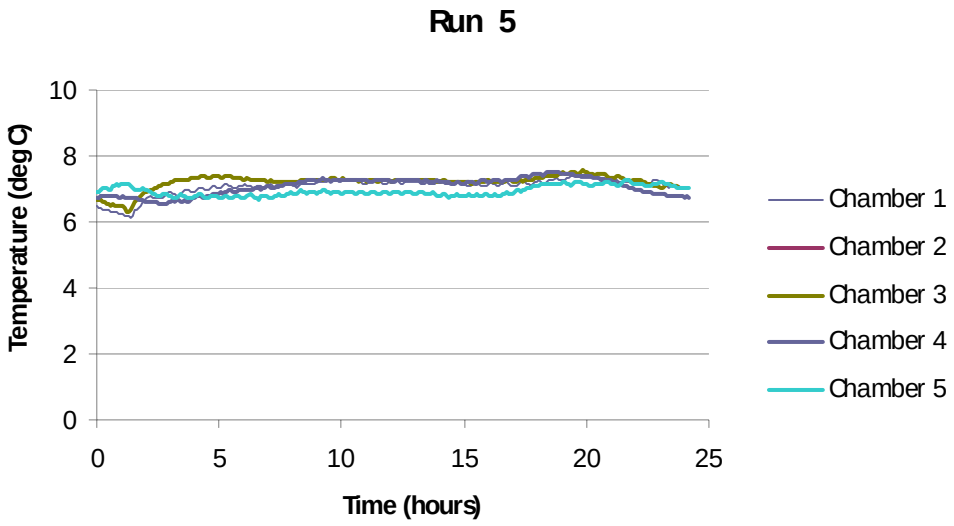
APPENDIX 5: TEMPERATURE PROFILES FROM EACH OZONE EXPERIMENT

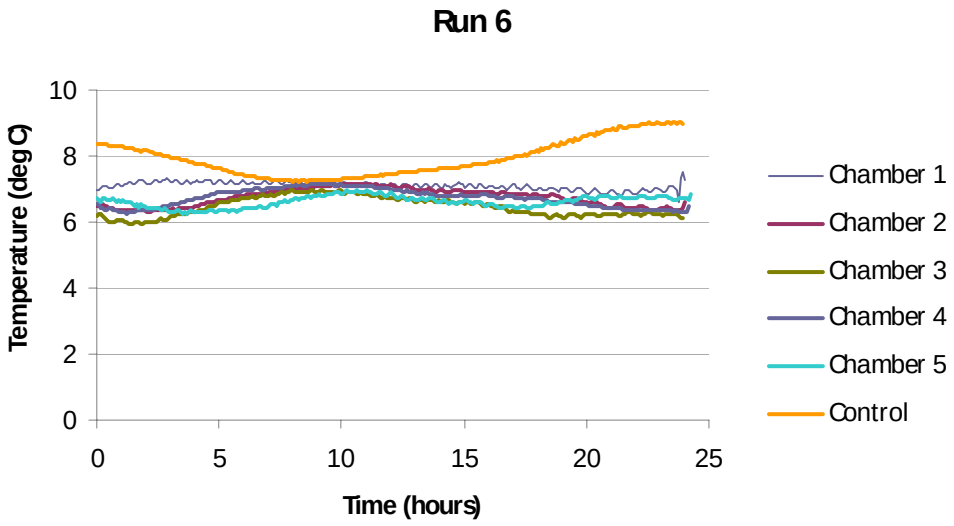


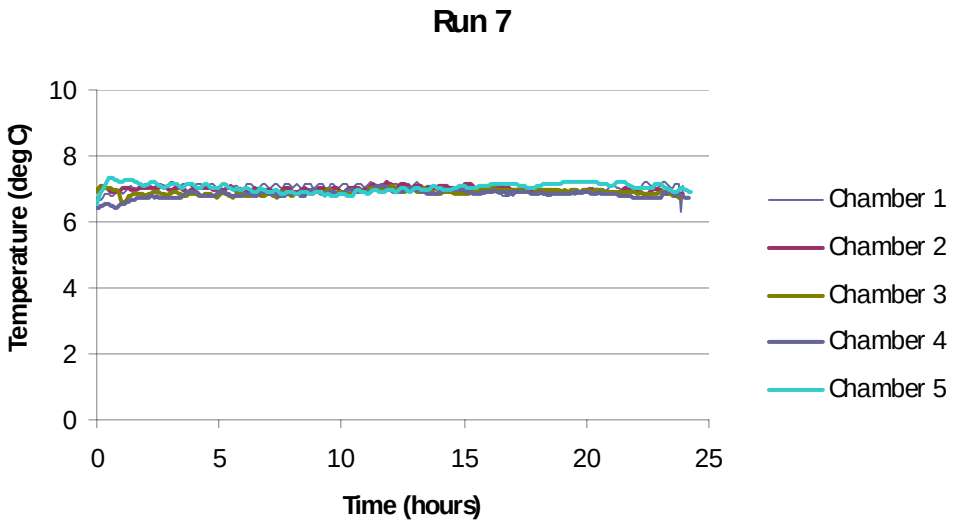



Run 3
Temperature (deg C)
Time (hours)
0
5
10
15
20
0
1
2
3
4
5
6
7
8
9
1 hour
1.5 hours
2 hours
3 hours
4 hours
8 hours
Control


Run 4
Temperature (deg C)
Time (hours)
0
5
10
15
20
0
1
2
3
4
5
6
7
8
9
1 hour
1.5 hours
2 hours
3 hours
4 hours
8 hours
Control


Run 5
Temperature (deg C)
Time (hours)
Chamber 1
Chamber 2
Chamber 3
Chamber 4
Chamber 5


Run 6
Temperature (deg C)
Time (hours)
Chamber 1
Chamber 2
Chamber 3
Chamber 4
Chamber 5
Control


Run 7
Temperature (deg C)
Time (hours)
Chamber 1
Chamber 2
Chamber 3
Chamber 4
Chamber 5

APPENDIX 6: FULL RESULTS OF MATERIALS TESTING

Tensile Testing

Tensile Properties of Plastic Film Type 5, Polypropylene Film (PP)

A full set of tensile testing results is presented for PP film (sample 5) as an example of what can be measured and determined (Tables A8.1-6). However, for the other samples, although full data sets were collected, only limited subsets of data are reported to minimise the amount of data in this progress report. The mean measured film thickness was 18.1 μm (nominal thickness 19 μm) before treatment and 31.4 μm after ozone treatment.

Within the unexposed samples, there is no statistically significant difference between the replicates measured in the same direction, either in the parallel direction (Table A6.1) or the perpendicular direction (Table A8.2) at a 95 percent confidence level.

In all following Tables: [a] ns – not significant; * - significant at the 95 percent confidence level, $P<0.05$; ** - significant at the 99 percent confidence level, $P<0.01$; and *** - significant at the 99.9 percent confidence level, $P<0.001$.

Table A6.1: Means and standard deviations of the three unexposed replicates in the parallel direction.

Measure	Rep. 1		Rep. 2		Rep. 3		Significance[a]
	Mean	SD	Mean	SD	Mean	SD	
Energy to break, MJ/m^3	48	60	11	2.0	20	23	ns
Break factor, kN/m	1.22	0.46	0.959	0.031	1.08	0.20	ns
Tensile strength, MPa	67.8	25.4	53.1	1.72	59.9	11.4	ns
Tensile strength at break, MPa	67.8	25.4	53.1	1.72	59.9	11.4	ns
Percent elongation at break, %	34	29	11	1.8	17	16	ns

Note: The maximum load was the same as the load at break

Table A6.2: Means and standard deviations of the three unexposed replicates in the perpendicular direction.

Measure	Rep. 1		Rep. 2		Rep. 3		Significance
	Mean	SD	Mean	SD	Mean	SD	
Energy to break, MJ/m^3	25	22	12	7.7	18	9.3	ns
Break factor, kN/m	2.72	0.908	1.85	0.788	2.56	0.510	ns
Tensile strength, MPa	150	50.2	103	43.6	142	28.3	ns
Tensile strength at break, MPa	150	50.2	103	43.6	142	28.3	ns
Percent elongation at break, %	11	4.8	7.5	3.7	8.6	2.43	ns

Note: There is very large variation among the five replicates tested for each sample. Such variation is very typical for plastic films, where a large variety of factors dictate film strengths/properties, hence the need for statistical analysis to look at the significance of changes after ozone treatment. There are no large errors introduced by other factors.

Within the exposed samples, there was no statistically significant difference between the replicates measured in the same direction, either in the parallel direction (Table A6.5) or the perpendicular direction (Table A6.4) at a 95 percent confidence level. This suggests that for this sample, the treatments carried out in the three different test chambers have yielded the same results.

Table A6.3. Means and standard deviations of the three exposed replicates in the parallel direction.

Measure	Rep. 1		Rep. 2		Rep. 3		Significance
	Mean	SD	Mean	SD	Mean	SD	
Energy to break, MJ/m^3	2.4	2.8	2.7	3.3	2.0	2.1	ns
Break factor, kN/m	0.617	0.242	0.657	0.181	1.01	0.416	ns
Tensile strength, MPa	19.6	7.69	20.9	5.76	32.1	13.2	ns
Tensile strength at break, MPa	19.6	7.69	20.9	5.68	32.1	13.2	ns
Percent elongation at break, %	3.6	2.6	5.1	3.6	2.6	0.96	ns

Table A6.4. Means and standard deviations of the three unexposed replicates in the perpendicular direction.

Measure	Rep. 1		Rep. 2		Rep. 3		Significance
	Mean	SD	Mean	SD	Mean	SD	
Energy to break, MJ/m^3	1.8	1.4	2.5	0.84	1.7	1.3	ns
Break factor, kN/m	1.02	0.264	1.17	0.114	0.860	0.346	ns
Tensile strength, MPa	32.3	8.38	37.3	3.61	27.4	11.0	ns
Tensile strength at break, MPa	32.3	8.38	37.3	3.61	27.4	11.0	ns
Percent elongation at break, %	3.3	1.9	3.2	0.85	3.9	1.9	ns

Within the unexposed samples:

- Break factor values measured in the parallel direction were statistically significantly higher than those measured in the perpendicular direction.
- Tensile strength and tensile strength at break values measured in the parallel direction were also statistically significantly higher than those measured in the perpendicular direction.
- Percent elongation at break values measured in the parallel direction were, on the other hand, statistically significantly lower than those measured in the perpendicular direction (Table A6.5).

Table A6.5. Means and standard deviations of the six unexposed samples grouped by directions.

Measure	Parallel direction		Perpendicular direction		Significance
	Mean	SD	Mean	SD	
Energy to break, MJ/m3	26	38	19	15	ns
Break factor, kN/m	1.09	0.292	2.38	0.798	***
Tensile strength, MPa	60.3	16.2	132	44.2	***
Tensile strength at break, MPa	60.3	16.2	132	44.2	***
Percent elongation at break, %	20	20	9.1	3.8	*

These differences no doubt reflect the anisotropic nature of these plastic films, i.e., different properties in different directions on the films.

Within exposed samples:

- Break factor values measured in the perpendicular direction were statistically significantly higher than those measured in the parallel direction.
- Tensile strength and tensile strength at break values measured in the perpendicular direction were also statistically significantly higher than those measured in the parallel direction (Table A6.6).

Table A6.6: Means and standard deviations of the six exposed samples grouped by directions.

Measure	Parallel direction		Perpendicular direction		Significance
	Mean	SD	Mean	SD	
Energy to break, MJ/m^3	2.4	2.6	2.0	1.2	ns
Break factor, kN/m	0.761	0.329	1.02	0.274	*
Tensile strength, MPa	24.2	10.5	32.3	8.73	*
Tensile strength at break, MPa	24.2	10.5	32.3	8.73	*
Percent elongation at break, %	3.8	2.7	3.4	1.5	ns

On the other hand, the differences in energy to break and percent elongation at break measurements were not found to be statistically significant.

Table A6.7 shows means and standard deviations of all samples grouped by treatments, i.e. six replicates each in exposed and unexposed groups. Unexposed samples had statistically significantly higher values than exposed samples for all the five measurements taken: energy to break, break factor, tensile strength, tensile strength at break and percent elongation at break.

Table A6.7: Means and standard deviations of the exposed and unexposed samples.

Measure	Exposed		Unexposed		Significance
	Mean	SD	Mean	SD	
Energy to break, MJ/m^3	2.2	2.0	22	28	***
Break factor, kN/m	0.888	0.325	1.73	0.881	***
Tensile strength, MPa	28.3	10.3	95.9	48.8	***
Tensile strength at break, MPa	28.3	10.3	95.9	48.8	***
Percent elongation at break, %	3.6	2.2	15	16	***

In conclusion, these data suggest that exposure to ozone of films of plastic of type 5 (PP) may lead to a reduction in tensile strength and extensibility and an increase in film thickness.

Tensile Properties of Plastic Film Types 1 to 4 and 6 and 7

For a film of plastic type 1 (sample 1, PET), data in Table A6.8 reveal that ozone exposure appeared to have little effect on the tensile properties of the material. The mean measured film thickness was 16.9 μm (nominal thickness 12 μm) before treatment and 13.1 μm after ozone treatment.

Table A6.8: Means and standard deviations of the exposed and unexposed samples for plastic type 1 film (PET).

Measure	Exposed		Unexposed		Significance
	Mean	SD	Mean	SD	
Energy to break, MJ/m^3	38	33	55	34	*
Break factor, kN/m	1.80	0.393	2.52	2.54	ns
Tensile strength, MPa	137	29.9	149	150	ns
Tensile strength at break, MPa	129	42.0	117	32.7	ns
Percent elongation at break, %	18	13	24	13	ns

In stark contrast, for a film of plastic type 2 (sample 2, PE-HD) ozone exposure appears to have had a very large effect on the tensile properties of the material. Exposed material was so brittle and damaged that its own mass or handling was enough to break it, and so no tensile testing was possible. For comparative purposes, means and standard deviations of the unexposed samples are given in Table A6.9. The mean measured film thickness was 16.5 μm (nominal thickness 20 μm) before treatment.

Table A6.9. Means and standard deviations of the unexposed samples for plastic type 2 film (PE-HD).

Measure	Unexposed	
	Mean	SD
Energy to break, MJ/m^3	99	56
Break factor, kN/m	0.571	0.137
Tensile strength, MPa	34.7	8.33
Tensile strength at break, MPa	23.5	11.3
Percent elongation at break, %	164	93

For a film of plastic type 3 (sample 3, PVC), data in Table A8.10 reveal that ozone exposure significantly reduced the tensile properties of the material. The mean measured film thickness was 10.7 μm (nominal thickness not known) before treatment and 11.7 μm after ozone treatment.

Table A6.10. Means and standard deviations of the exposed and unexposed samples for plastic type 3 film (PVC).

Measure	Exposed		Unexposed		Significance
	Mean	SD	Mean	SD	
Energy to break, MJ/m^3	5.2	2.8	8.4	4.4	***
Break factor, kN/m	0.184	0.050	0.192	0.029	ns
Tensile strength, MPa	15.7	4.27	18.0	2.73	***
Tensile strength at break, MPa	5.97	3.79	11.4	4.19	***
Percent elongation at break, %	22	9.9	39.9	20.6	***

For a film of plastic type 4 (sample 4, PE-LD), data in Table A6.11 reveal that ozone exposure significantly reduced the tensile properties of the material. The mean measured film thickness was 24.8 μm (nominal thickness 30 μm) before treatment and 31.7 μm after ozone treatment.

Table A6.11. Means and standard deviations of the exposed and unexposed samples for plastic type 4 film (PE-LD).

Measure	Exposed		Unexposed		Significance
	Mean	SD	Mean	SD	
Energy to break, MJ/m^3	1.2	0.51	172	87	***
Break factor, kN/m	0.403	0.085	0.609	0.219	***
Tensile strength, MPa	12.7	2.67	24.6	8.82	***
Tensile strength at break, MPa	12.7	2.67	16.5	9.25	*
Percent elongation at break, %	3.3	0.80	275	145	***

For a film of plastic type 6 (sample 6, PS), data in Table A6.12 reveal that ozone exposure significantly reduced some of the tensile properties of the material. The mean measured film thickness was 29.2 µm (nominal thickness unknown) before treatment and 31.5 µm after ozone treatment.

Table A6.12. Means and standard deviations of the exposed and unexposed samples for plastic type 6 film (PS).

Measure	Exposed		Unexposed		Significance
	Mean	SD	Mean	SD	
Energy to break, MJ/m^3	2.6	1.3	3.3	2.6	ns
Break factor, kN/m	1.86	0.390	1.91	0.447	ns
Tensile strength, MPa	59.2	12.4	65.4	15.3	*
Tensile strength at break, MPa	59.1	12.4	64.9	14.9	*
Percent elongation at break, %	2.6	0.54	2.6	0.71	ns

For a film of plastic type 7 (sample 7, nylon), data in Table A6.13 reveal that ozone exposure did not significantly alter the tensile properties of the material. The mean measured film thickness was 16.6 µm (nominal thickness 10 µm) before treatment and 15.8 µm after ozone treatment.

Table A6.13. Means and standard deviations of the exposed and unexposed samples for plastic type 7 film (nylon).

Measure	Exposed		Unexposed		Significance
	Mean	SD	Mean	SD	
Energy to break, MJ/m^3	28	20	25	18	ns
Break factor, kN/m	1.50	0.436	1.50	0.429	ns
Tensile strength, MPa	94.8	27.5	90.1	25.8	ns
Tensile strength at break, MPa	94.6	27.2	89.6	25.7	ns
Percent elongation at break, %	18	9.6	17	9.2	ns

In addition, within the exposed samples of PET, PS and nylon there were no statistically significant differences between the replicates measured in the same directions, suggesting that the sample treatments carried out in the three different chambers yielded the same results. For the other exposed samples (PVC, PP, PE-LD), there were statistically significant differences between the replicates in the same directions and it is unclear from where these originate,

assuming treatment conditions in the three treatment vessels were identical, as other materials test results suggested. The differences could have arisen from local inhomogeneity in the films of these products, as similar statistically significant differences were often observed in their exposed counterparts.

Films of PE-HD, PE-LD, PS and nylon also showed some degree of anisotropy similar to that observed for the bidirectional PP film. Statistically significant differences were observed in various tensile properties between the parallel and perpendicular directions in unexposed and, in some cases, exposed samples.

Tensile Properties of Rubber Samples

Tensile properties of the two rubber samples tested are summarised in Tables A6.14 and A6.15.

Table A6.14: Means and standard deviations of the exposed and unexposed natural rubber samples.

Measure	Exposed		Unexposed		Significance
	Mean	SD	Mean	SD	
Tensile strength, MPa	24.6	1.33	25.2	1.05	ns
Breaking elongation, %	412	21	447	15	**

Table A6.15. Means and standard deviations of the exposed and unexposed EPDM rubber samples.

Measure	Exposed		Unexposed		Significance
	Mean	SD	Mean	SD	
Tensile strength, MPa	10.5	0.238	10.6	0.152	ns
Breaking elongation, %	173	16	165	12	ns

The breaking elongation of the exposed natural rubber sample was significantly lower than that of the unexposed rubber sample, but no such differences were observed for the EPDM rubber samples. There were no significant differences between the replicates within the exposed and unexposed samples for each of the two different types of rubbers, again suggesting that the ozone treatments in the three treatment vessels appeared to be uniform.

Burst Strength of Corrugated Cardboard Samples

Burst strengths of the two cardboard samples tested are summarised in Table A6.16.

Table A6.16: Means and standard deviations of the exposed and unexposed cardboard samples.

	Exposed		Unexposed		Significance
Burst strength, kPa	Mean	SD	Mean	SD	
Storage box	2120	315	2210	245	ns
Copier paper box	638	44	789	108	**

The burst strength of the exposed thinner corrugated cardboard sample was significantly lower than that of the unexposed sample but no such differences were observed for the thicker sample. There were no significant differences between the replicates within the exposed and unexposed samples for each of the two different types of corrugated cardboard. These data suggested that the ozone treatments in the three treatment vessels were uniform.

Visual inspection

The following are observations made from the visual inspection of each sample, with any differences observed between the ozone-exposed specimen and the unexposed control specimen.

C1

The inside painted surface of C1 was off-white in colour and slightly glossy with a smooth texture (Figure A6.1). C1 differed from the inside painted surface of C2 and C3 in that the paint was off-white in colour instead of grey. There seemed to be a very slight change in colour after exposure to ozone, as the colour of the ozone-exposed specimen appeared to have a less orange tone than the unexposed control. Cracks were apparent around the corroded region in both the ozone exposed and unexposed control. There were no other cracks or blisters observed with the unaided eye.

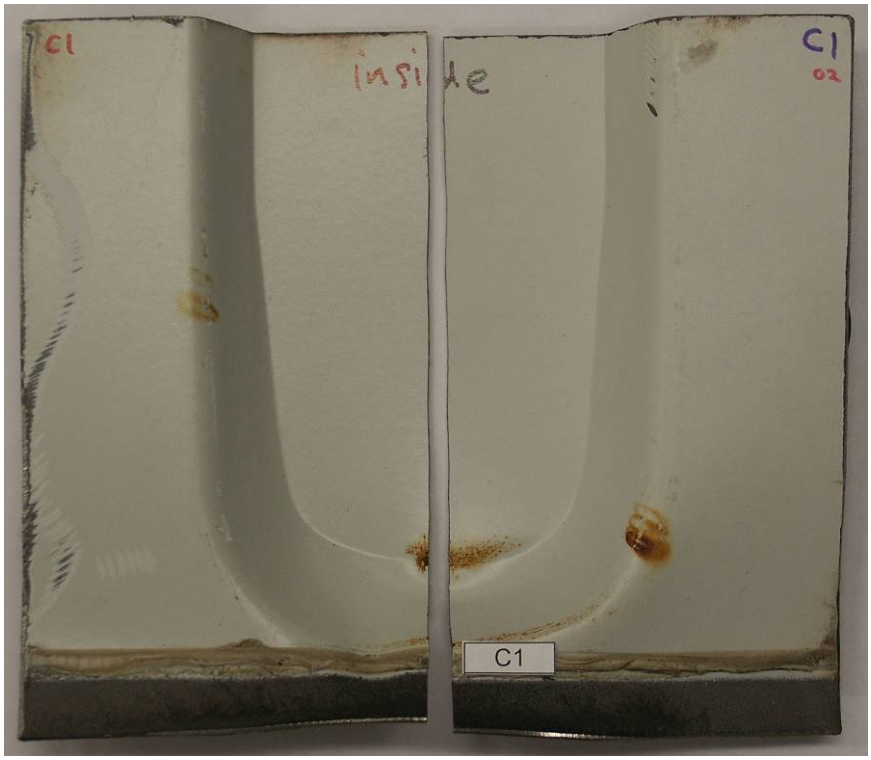


Figure A6.1: Photograph of C1. Unexposed reference specimen (left), and ozone-exposed specimen (right).

C2

The inside painted surface of C2 was light-grey and matt and the surface was textured (Figure A6.2). There was no detectable change in colour between the ozone-exposed sample and the unexposed control. There were some scratches present on the paint, but these scratches were thought to have been caused by handling. There were no blisters or hairline cracks observed with the unaided eye.

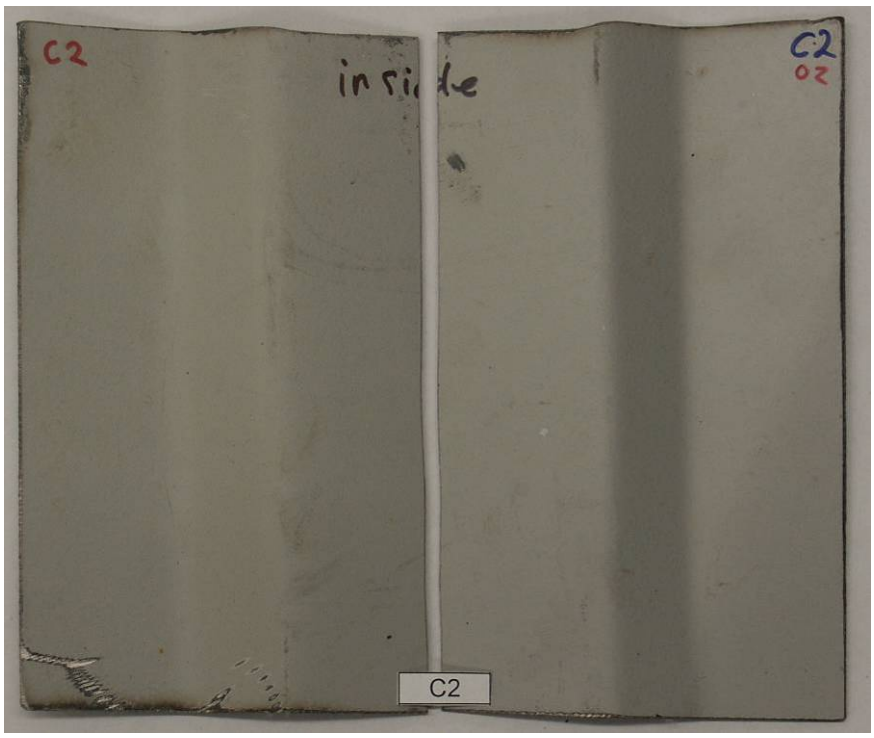


Figure A6.2: Photograph of C2. Unexposed reference specimen (left), and ozone-exposed specimen (right).

C3

The inside painted surface of C3 was similar to the inside painted surface of C2. It was light grey and matt and the surface was textured (Figure A6.3). There was black spotting present, which was concentrated mainly on the unexposed control sample because of sample selection. It was not possible to remove these black spots when they were rubbed with a tissue. There was no clear difference in colour between the ozone-exposed sample and the unexposed control. There were no cracks or blisters observed with the unaided eye.

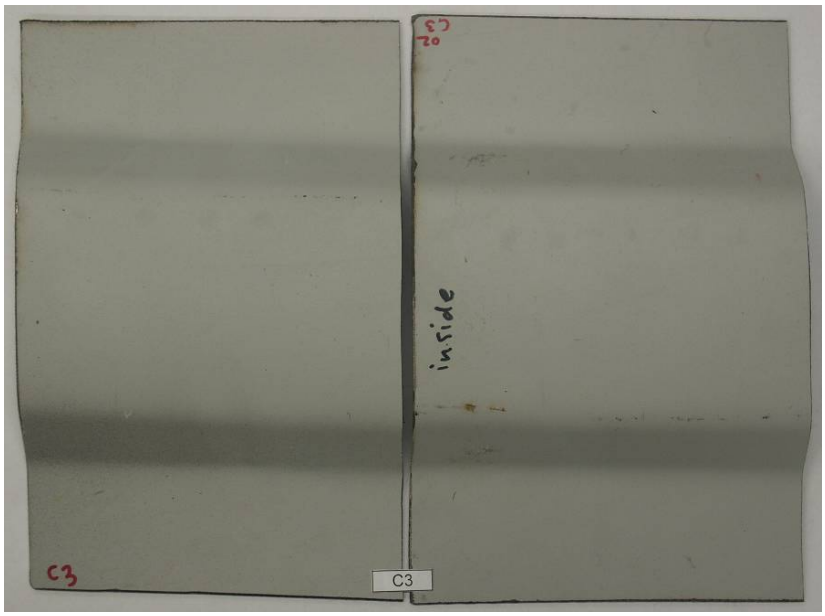


Figure A6.3. Photograph of C3. Unexposed reference specimen (left), and ozone-exposed specimen (right).

UC1

UC1 was light grey with darker grey surface speckling. There was a patch that was darker in colour, as well as some light surface rust (Figure A6.4). The UC1 sample was thinner than the UC2 and UC3 samples. There was no significant difference observed between the unexposed control and the ozone-exposed specimen.

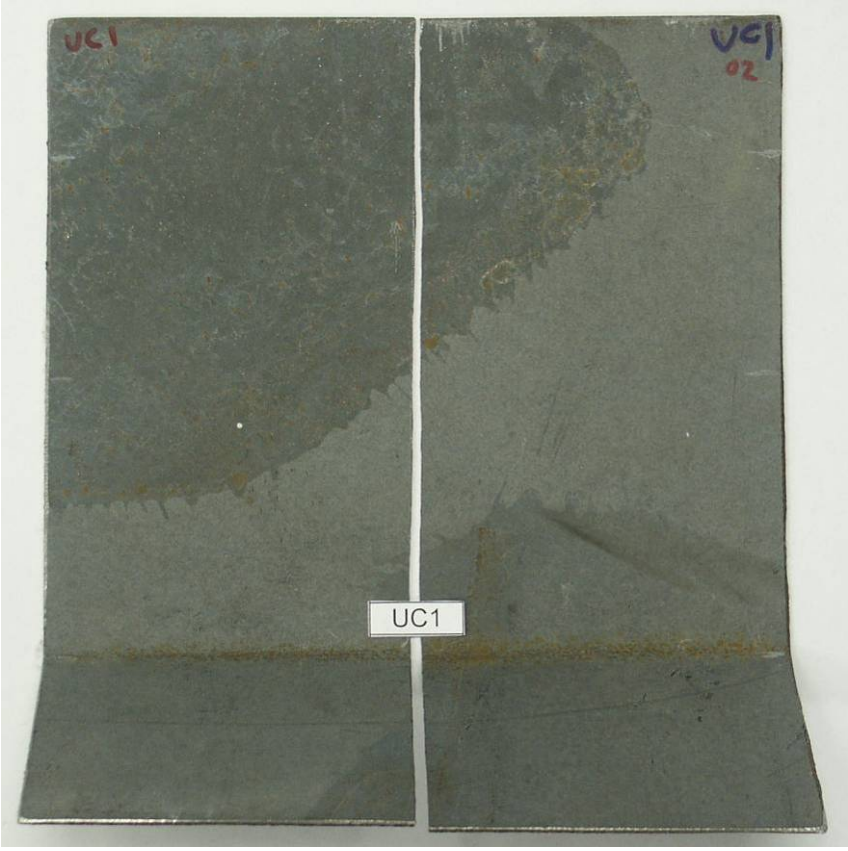


Figure A6.4. Photograph of UC1. Unexposed reference specimen (left), and ozone-exposed specimen (right)

UC2

UC2 had a grey and black spotted surface and some patches of surface rust (Figure A8.5). There were also some blemishes and scratching present, but these features were thought to be more likely to have been caused by handling. There was no significant difference observed between the unexposed control and the ozone-exposed specimen.

Figure A6.5: Photograph of UC2. Unexposed reference specimen (left), and ozone-exposed specimen (right).

UC3

UC3 was very similar to UC2, with a grey and black spotted surface and some patches of light surface rust (Figure A6.6). There were some scratches present, but these scratches were thought to be more likely to have been caused by handling. There was no significant difference observed between the unexposed control and the ozone-exposed specimen.

Figure A6.6: Photograph of UC3. Unexposed reference specimen (left), and ozone-exposed specimen (right).

Microscopic inspection

C1

Figures A6.7 and A6.8 are microscope images of the inside painted surface of C1 with and without exposure to ozone. The images show a raised feature that was present on both the ozone-exposed and unexposed control specimens. Because these features were present on both the exposed and unexposed surface, they were not considered results of the ozone treatment. The regions surrounding the feature were smooth and there were no significant differences observed between the surfaces of the exposed and unexposed specimens, apart from some superficial scratches apparent on the unexposed control specimen at 40 x magnification (Figure A8.7, right).

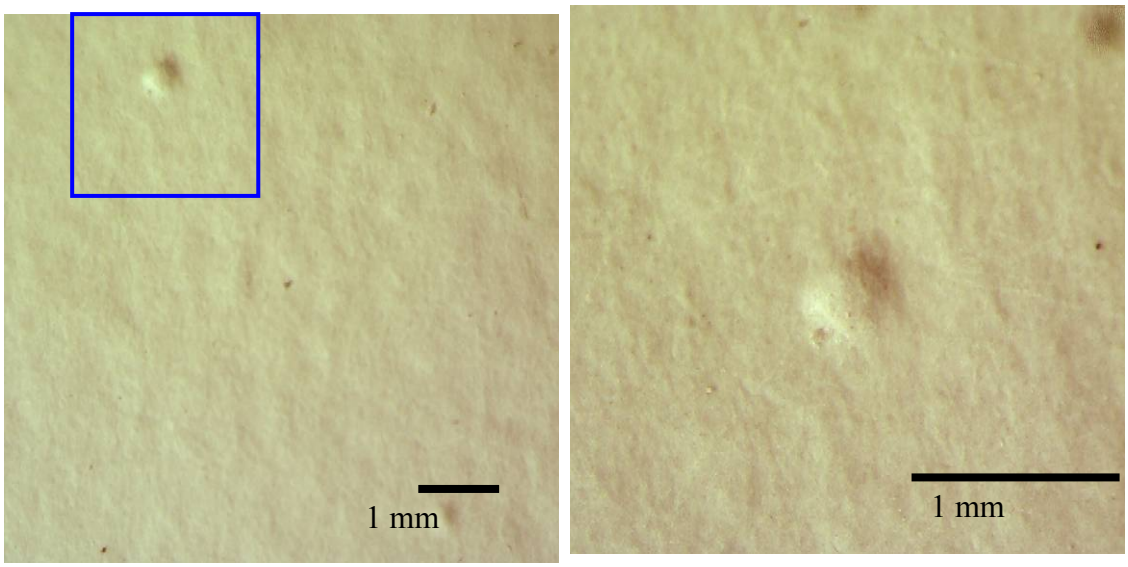


Figure A6.7: Raised feature on C1 unexposed control specimen. 16 x magnification (left) and region highlighted in blue box at 40 x magnification (right).

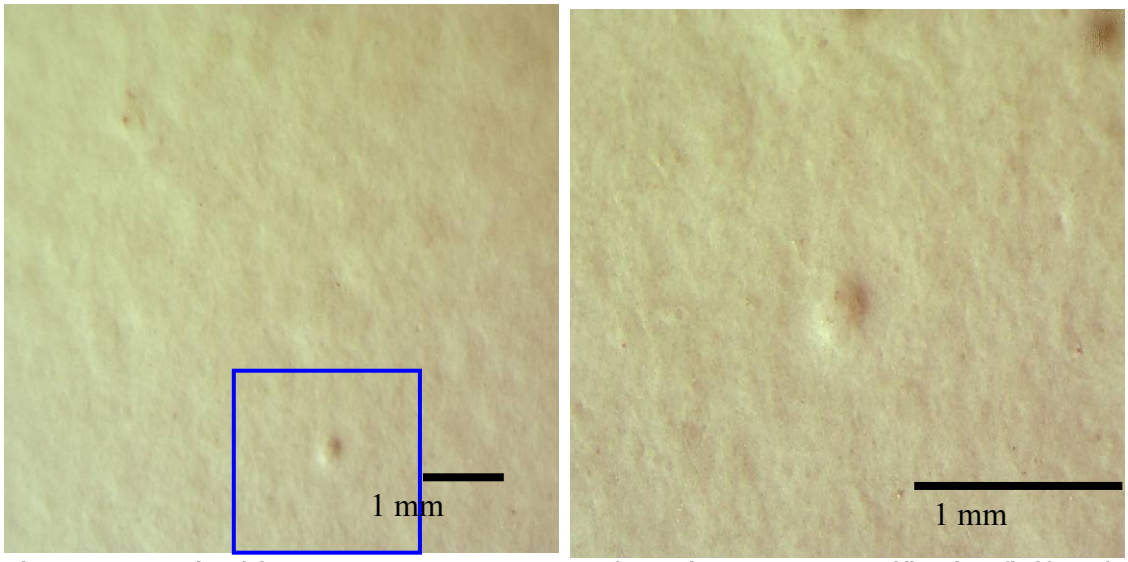


Figure A6.8. Raised feature on C1 ozone-exposed specimen. 16 x magnification (left) and region highlighted in blue box at 40 x magnification (right).

Figure A6.9 shows cracking around a corroded region on the inside painted surface of C1. Comparisons between the unexposed control and the ozone-exposed specimen indicated that cracking was present in both specimens and was therefore not a result of ozone exposure. A visual scan of the entire surface of both samples did not reveal any additional cracking away from the corroded regions shown in Figure A8.9.

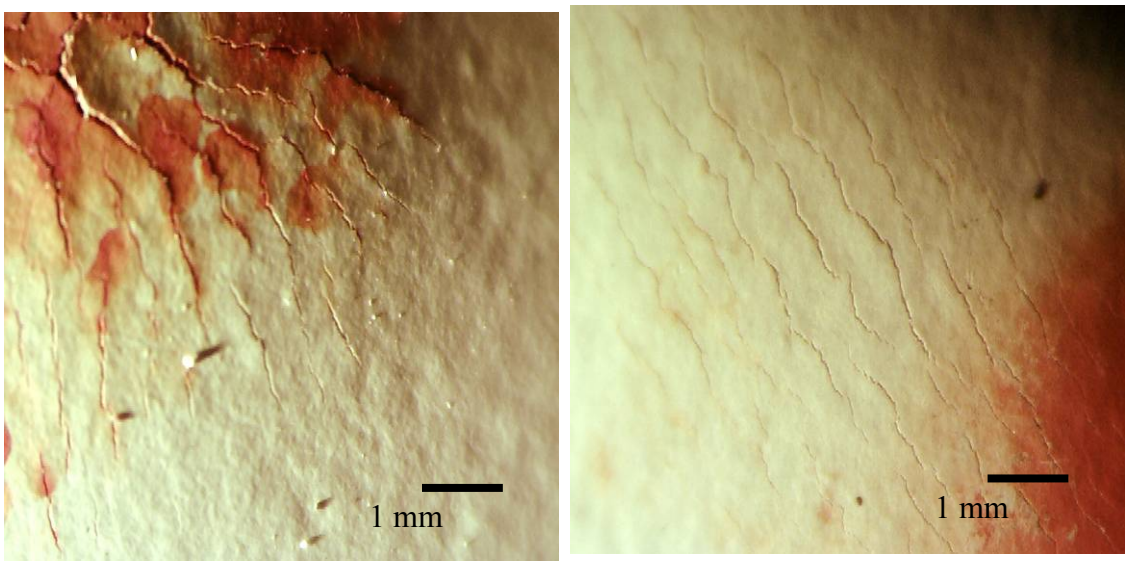


Figure A6.9: Cracking around corroded region on C1. Unexposed control specimen (left) and ozone-exposed specimen (right). 16 x magnification.

Figure A6.10 shows a crater feature on the inside painted surface of the C1 ozone-exposed specimen. This single crater feature was observed only on the ozone-exposed specimen. No crater feature(s) was observed on the unexposed specimen of C1. Although this crater feature was observed only on the ozone-exposed specimen, it is likely be a result of the manufacturing process and not a product of exposure to ozone.

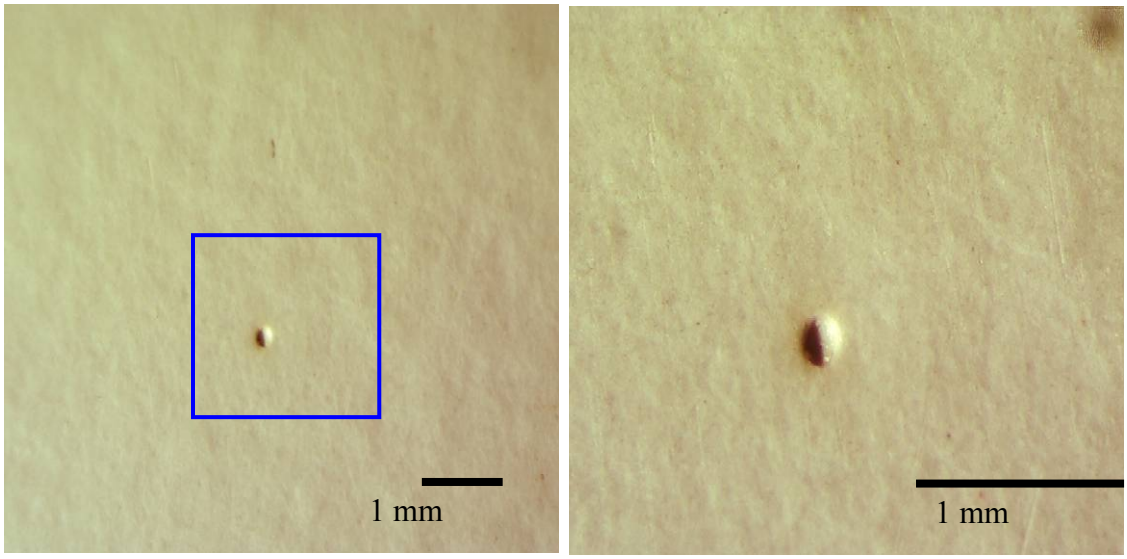


Figure A6.10. Crater feature on C1 ozone-exposed specimen. 16 x magnification (left) and region highlighted in blue box at 40 x magnification (right).

C2

Figures A6.11 and A6.12 are microscope images of the inside painted surface of C2 with and without exposure to ozone. The images show a raised feature that was present on both the ozone-exposed and unexposed control specimens and was therefore not a result of ozone exposure. The regions surrounding the feature were textured and there were no significant visual differences observed between the surfaces of the exposed and unexposed specimens. The surface texture of C2 was rougher than that of C1.

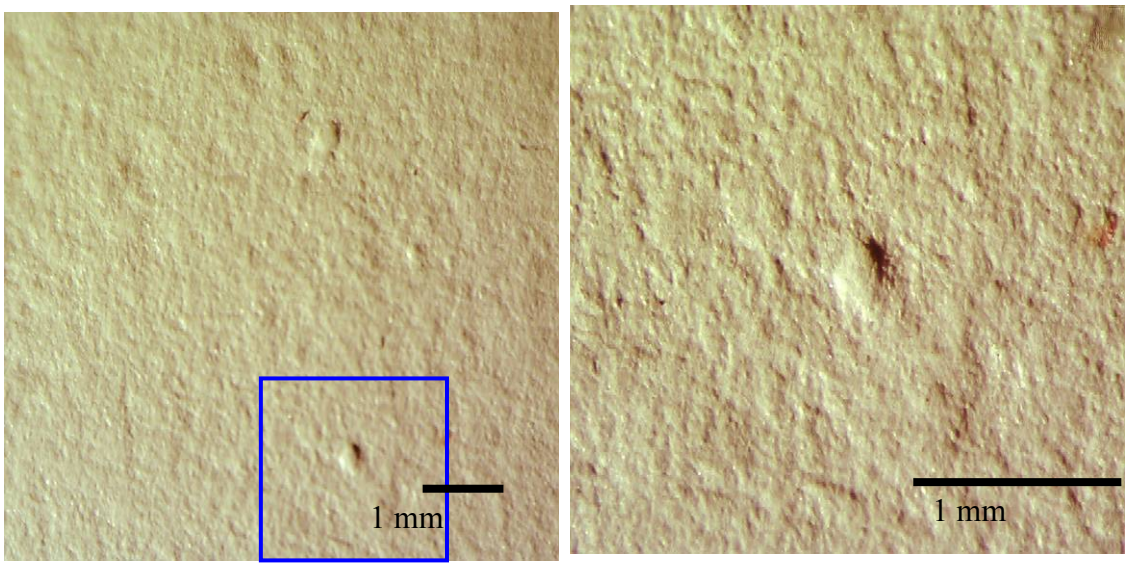


Figure A6.11: Raised feature on C2 unexposed control specimen. 16 x magnification (left) and region highlighted in blue box at 40 x magnification (right).

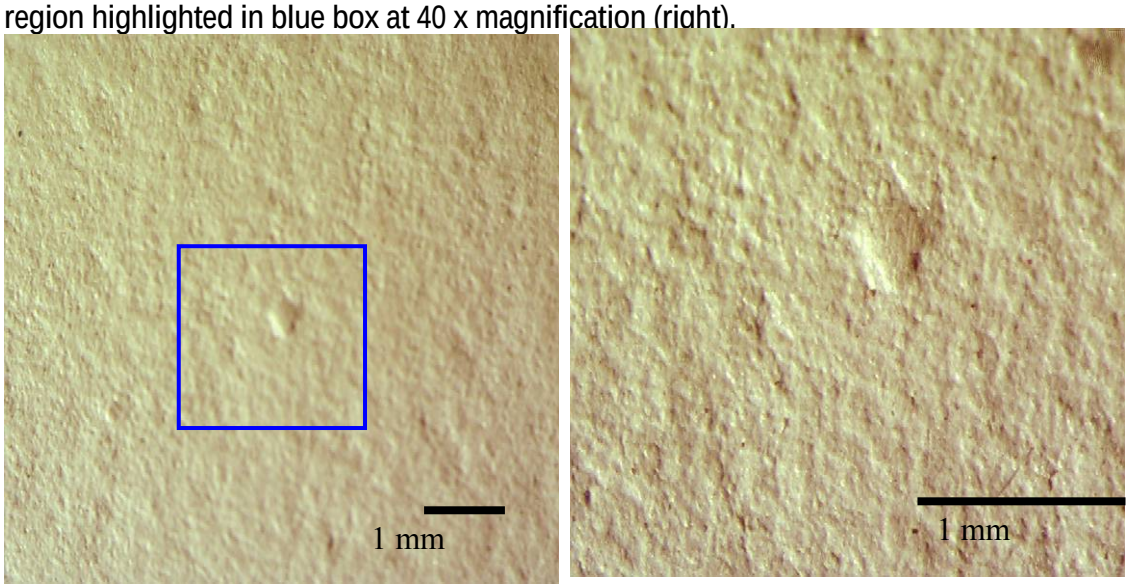


Figure A6.12: Raised feature on C2 ozone-exposed specimen. 16 x magnification (left) and region highlighted in blue box at 40 x magnification (right).

Figure A6.13 shows a series of cracks on the ozone-exposed inside painted surface of the C2 specimen. These cracks were unidirectional and were concentrated near a region of deformation on the sample and therefore could be stress-induced cracks, rather than a direct result of exposure to ozone.

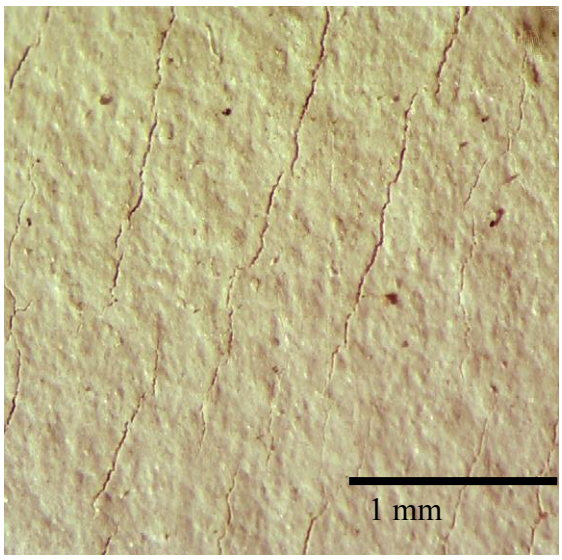


Figure A6.13. Cracking on C2 ozone-exposed specimen near deformed region, 40 x magnification.

C3

Figures A6.14 and 15 are microscope images of the inside painted surface of C3 with and without exposure to ozone. The images show a dark feature that was present on both the unexposed control and the ozone-exposed specimen. The composition of these features is unknown, but they were observed over a large area of both the exposed and unexposed specimens. There was no significant difference observed between the unexposed control sample and the ozone-exposed sample.

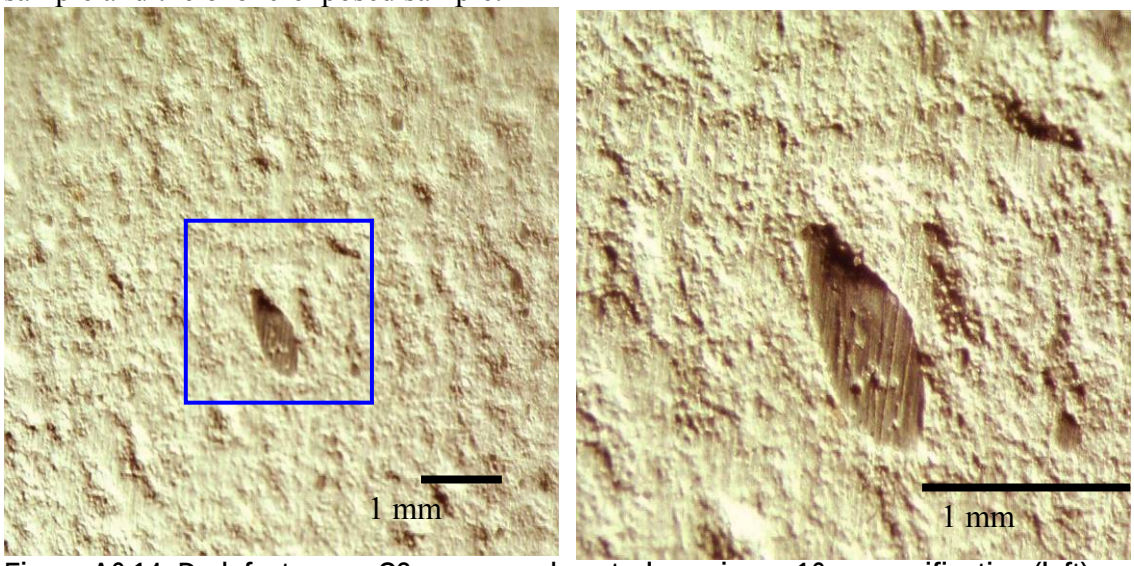


Figure A6.14: Dark feature on C3 unexposed control specimen. 16 x magnification (left) and region highlighted in blue box at 40 x magnification (right).

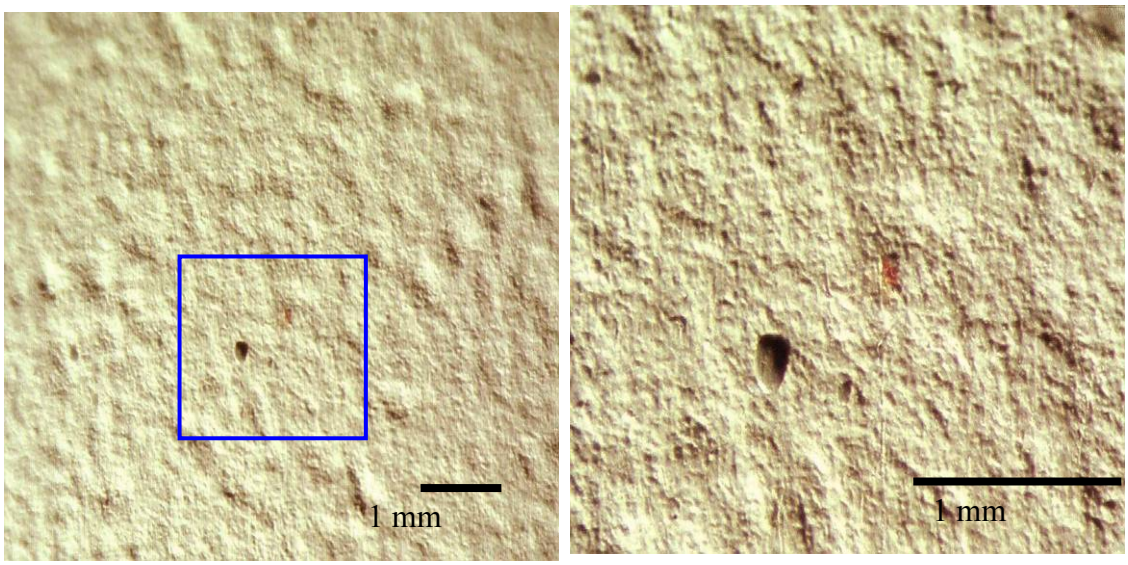


Figure A6.15: Dark feature on C3 ozone-exposed specimen. 16 x magnification (left) and region highlighted in blue box at 40 x magnification (right).

UC1

Figures A6.16 and 17 are microscope images of UC1 with and without exposure to ozone. The images show the surface to be very textured and covered in craters. There was no significant difference observed between the unexposed control sample and the ozone-exposed sample.

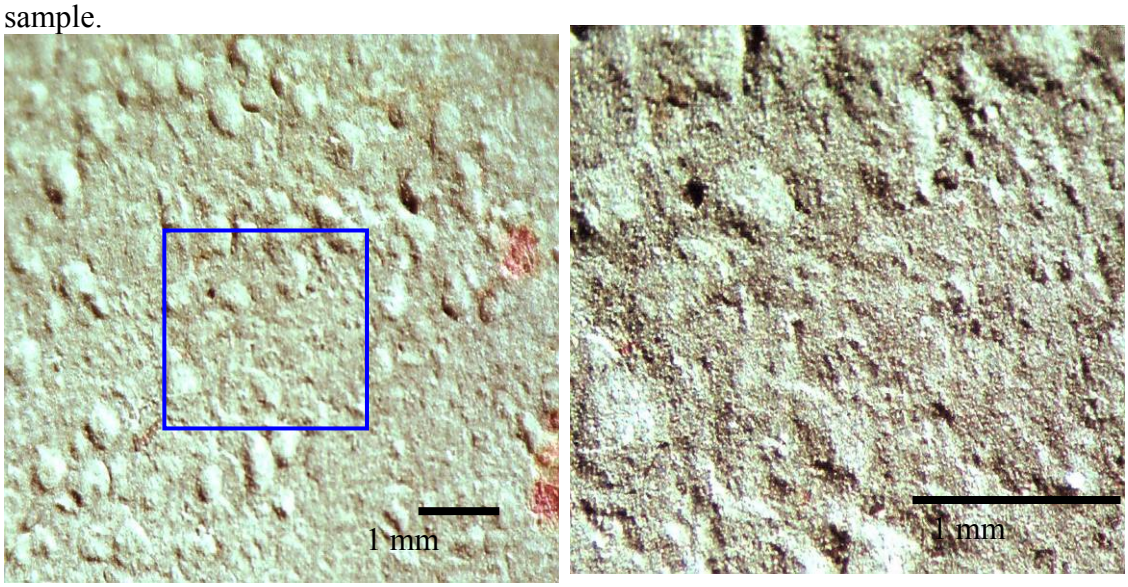


Figure A6.16: Random region on UC1 unexposed control specimen. 16 x magnification (left) and region highlighted in blue box at 40 x magnification (right).

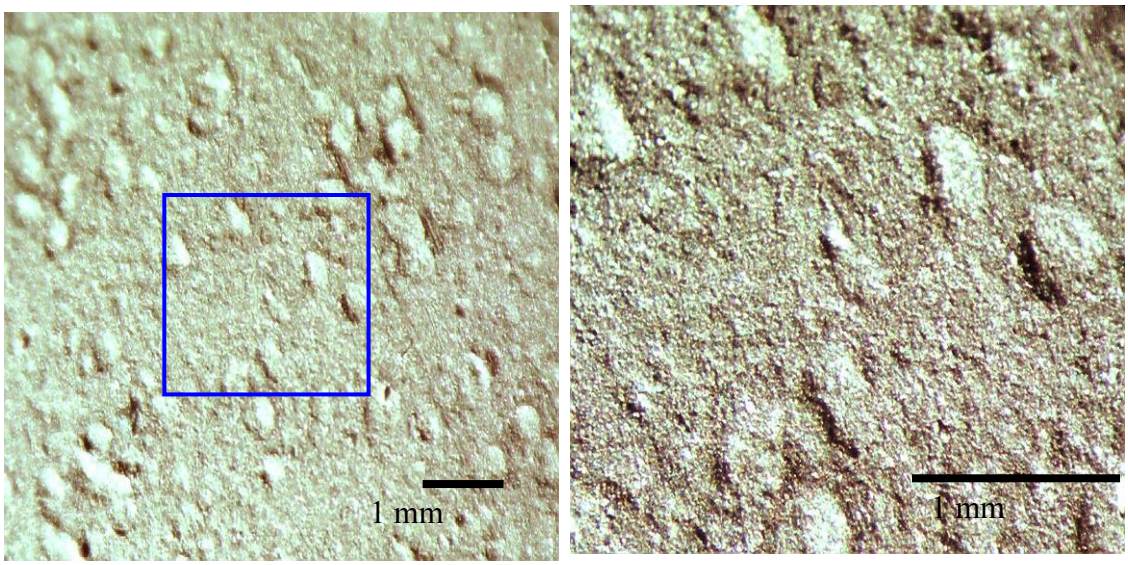


Figure A6.17: Random region on UC1 ozone-exposed specimen. 16 x magnification (left) and region highlighted in blue box at 40 x magnification (right).

UC2

Figures A6.18 and 19 are microscope images of UC2 with and without exposure to ozone. The images show the surface to be textured and covered in deep craters. The craters on UC2 were much deeper and were more extensive than on sample UC1. Figure A6.19 shows a region with what appears to be a small zone of corrosion. The surface around this region appeared to be quite porous. However in general, there was no significant difference observed between the unexposed control sample and the ozone-exposed sample.

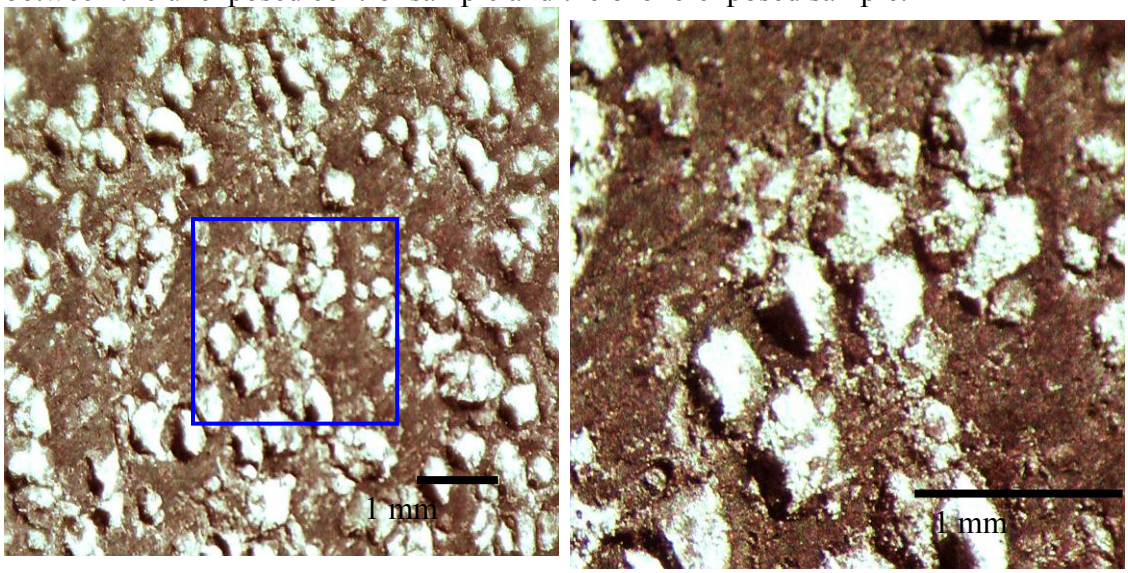


Figure A6.18: Random region on UC2 unexposed control specimen. 16 x magnification (left) and region highlighted in blue box at 40 x magnification (right).

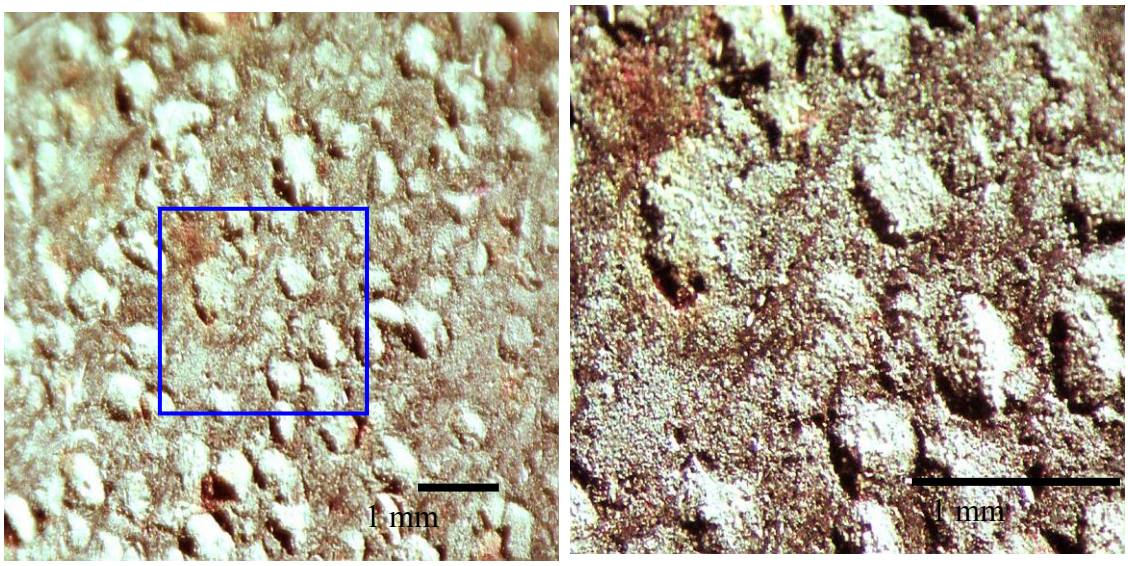


Figure A6.19: Random region on UC2 ozone-exposed specimen. 16 x magnification (left) and region highlighted in blue box at 40 x magnification (right).

UC3

Figures A6.20 and 21 are microscope images of UC3 with and without exposure to ozone. The images show the surface to be textured and covered in craters. The craters of UC3 were much deeper and were more extensive than those on sample UC1. There was no significant difference observed between the unexposed control sample and the ozone-exposed sample.

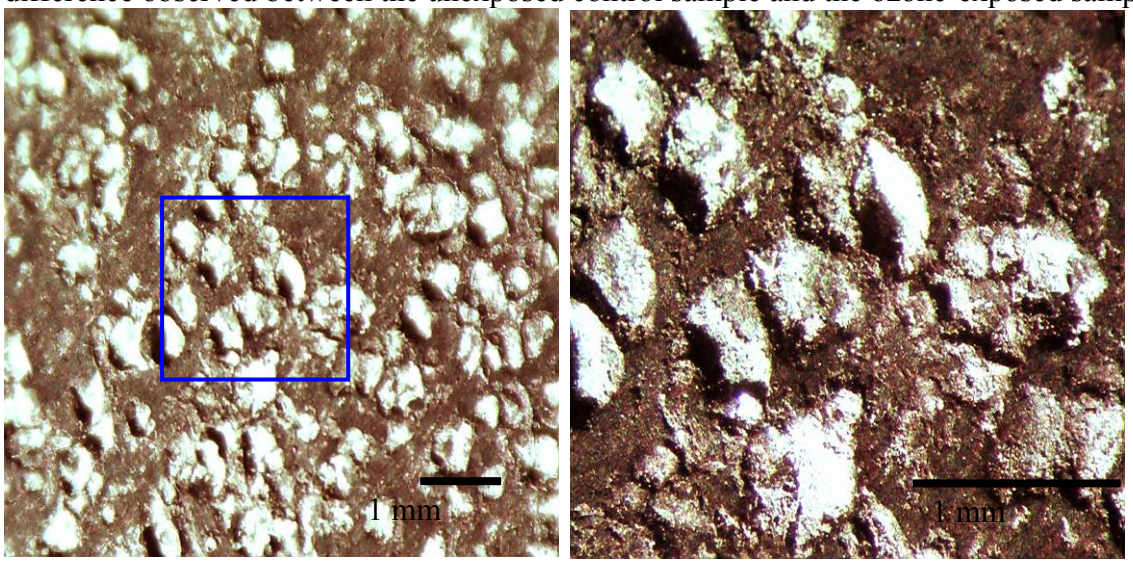


Figure A6.20: Random region on UC3 unexposed control specimen. 16 x magnification (left) and region highlighted in blue box at 40 x magnification (right).

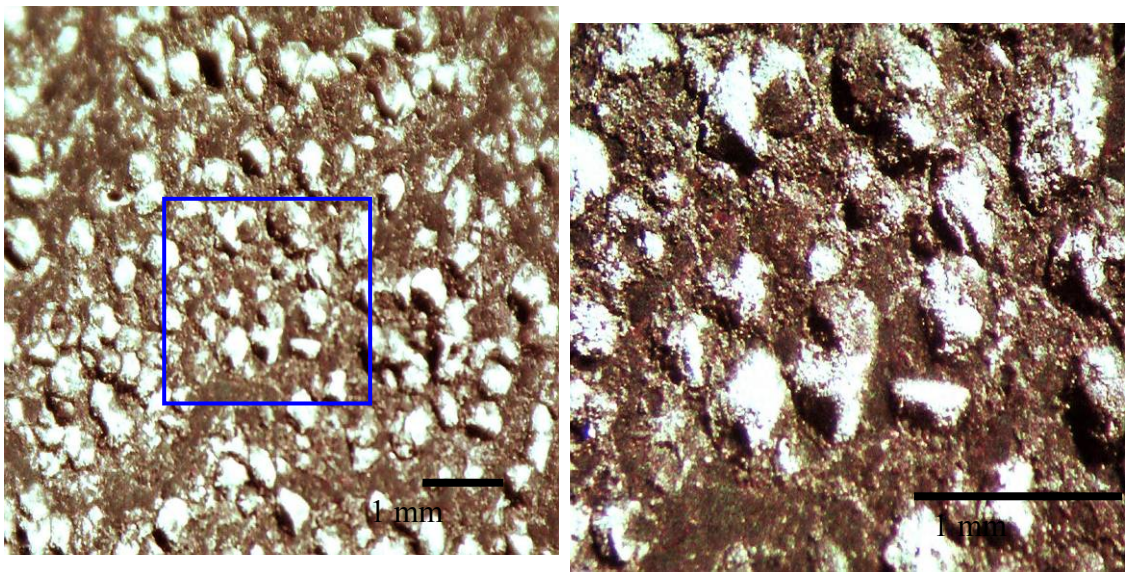


Figure A6.21: Random region on UC3 ozone-exposed specimen. 16 x magnification (left) and region highlighted in blue box at 40 x magnification (right).

APPENDIX 7: CALCULATION OF TENSILE PROPERTIES (FROM: ASTM D882 – 02 STANDARD TEST FOR TENSILE PROPERTIES OF THIN PLASTIC SHEETING)

Breaking Factor (nominal) shall be calculated by dividing the maximum load by the original minimum width of the specimen. The result shall be expressed in force per unit of width, usually Newtons per metre (or pounds per inch) of width and reported to three significant figures. The thickness of the film shall always be stated to the nearest 0.0025 mm (0.0001 in.).

Example - Breaking Factor = 1.75 kN/m (10.0 Ibf/in.) of width for 0.1300-mm (0.0051-in.) thickness.

NOTE 1. This method of reporting is useful for very thin films (0.13 mm (0.005 in.) and less) for which breaking load may not be proportional to cross-sectional area and whose thickness may be difficult to determine with precision. Furthermore, films that are in effect laminar because of orientation, skin effects, nonuniform crystallinity, etc., have tensile properties disproportionate to cross-sectional area.

Tensile Strength (nominal) shall be calculated by dividing the maximum load by the original minimum cross-sectional area of the specimen. The result shall be expressed in force per unit area, usually megaPascals (or pounds-force per square inch). This value shall be reported to three significant figures.

NOTE 2. When tear failure occurs, so indicate and calculate results based on load and elongation at which tear initiates, as reflected in the load-deformation curve.

Tensile Strength at Break (nominal) shall be calculated in the same way as the tensile strength, except that the load at break shall be used in place of the maximum load (Note 2 and Note 3).

NOTE 3. In many cases, tensile strength and tensile strength at break are identical.

Percent Elongation at Break shall be calculated by dividing the extension at the moment of rupture of the specimen by the initial gauge length of the specimen and multiplying by 100. When gauge marks or extensometers are used to define a specific test section, only this length shall be used in the calculation; otherwise the distance between the grips shall be used, reported to two significant figures (Note 2).

Tensile Energy to Break, where applicable, shall be calculated by integrating the energy per unit volume under the stress-strain curve or by integrating the total energy absorbed and dividing it by the volume of the original gauge region of the specimen. This may be done directly during the test by an electronic integrator, or subsequently by computation from the area of the plotted curve. The result shall be expressed in energy per unit volume, usually in megajoules per cubic metre (or inch-pounds-force per cubic inch). This value shall be reported to two significant figures.

For each series of tests, the arithmetic mean of all values obtained shall be calculated to the proper number of significant figures.

The standard deviation (estimated) shall be calculated as follows and reported to two significant figures:

$$s = \sqrt{(\Sigma X^2 - n\bar{X}^2)/(n-1)}$$

where:

s estimated standard deviation
X value of a single observation
n number of observations
$\bar{X}$ arithmetic mean of the set of observations.