Australian Pesticides &
Veterinary Medicines Authority

The reconsideration of approvals of the active constituent diazinon, registrations of products containing diazinon and approval of their associated labels

Part 2

Preliminary Review Findings

Volume 2 of 2

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GLOSSARY AND UNITS OF MEASURE

ACPH	Advisory Committee on Pesticides and Health
ADI	Acceptable daily intake
AJR	Automatic jetting race
APVMA	Australian Pesticides & Veterinary Medicines Authority
ARfD	Acute reference dose
Avcare	National Association for Crop Production and Animal Health. From 1 January 2006, Avcare Limited has been known as CropLife Australia Limited. CropLife Australia represents the plant science industry and is responsible for the crop protection and crop biotechnology aspects of Avcare. The animal health aspects will be managed by Animal Health Alliance (Australia) Ltd.
AWI	Australian Wool Innovation Limited
ChE	Cholinesterase
CRP	Chemistry and Residues Program of the APVMA
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DEH	Department of the Environment and Heritage
DETP	Diethylthiophosphate
DT50	Time for 50% of the substance to dissipate
EbC50	The concentration of a test substance which results in a 50% inhibition of biomass in an algal test
EC	Emulsifiable concentrate
EC50	The concentration of a test substance which results in 50% of the test organism being adversely affected ie both mortality and sub-lethal effects
FAISD	First Aid Instructions and Safety Directions
FAISD Handbook	Handbook of First Aid Instructions, Safety Directions, Warning Statements and General Safety Precautions for Agricultural and Veterinary Chemicals
GAP	Good agricultural practice
IRED	Interim reregistration eligibility decision
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Koc	Sorption/desorption coefficient, normalised to organic carbon content
Kow	n-Octanol/water partitioning coefficient
LC0	The concentration of a test substance at which no effect occurred
LC50	The concentration of a test substance which results in a 50% mortality of the test species.
LOEC	Lowest observed effect concentration ie the lowest test concentration at which some adverse effect occurs
LOEL	Lowest observed effect level
MAC	Maximum allowable concentration
MATC	Maximum acceptable toxicant concentration
MOE	Margin of exposure
Monotepp	O,S-TEPP
MRL	Maximum residue limit
NEDI	National estimated daily intake
NESTI	National estimated short-term intake

NOAEL	No observed adverse effect level
NOEC	No observed effect concentration ie the highest test concentration at which no adverse effect is observed
NOEL	No observed effect level
NOHSC	National Occupational Health and Safety Commission, now the Office of the Australian Safety and Compensation Council (OASCC)
NRA	National Registration Authority for Agricultural and Veterinary Chemicals, now APVMA
NRS	National Registration Scheme
OASCC	Office of the Australian Safety and Compensation Council
OCS (OHS)	Occupational Health and Safety area within the OCS, conducts assessments previously undertaken by the NOHSC prior to it becoming the OASCC
OCS	Office of Chemical Safety
OP	Organophosphate
O,S-TEPP	O,O,O',O'-tetraethyl-monothiopyrophosphate, also known as monotepp
PMRA	Pest Management Regulatory Agency (Canada)
PNEC	Predicted no effect concentration
PPE	Personal protective equipment
PRF	Preliminary Review Findings
S,S-TEPP	O,O,O',O'-tetraethyl dithiopyrophosphate, also known as sulfotepp
Sulfotepp	S,S-TEPP
TLm	Median level toxic concentration ie the test concentration at which 50% effect occurs
USEPA	United States Environmental Protection Agency

Time

d	Day
h	Hour
min	Minute
mo	Month
wk	Week
s	Second
yr	Year

Length

cm	Centimetre
m	Metre
µm	Micrometre
mm	Millimetre
nm	Nanometre

Volume

ML	Megalitre
L	Litre
mL	Millilitre
µL	Microlitre

Weight

bw	Body weight
g	Gram
kg	Kilogram
µg	Microgram
mg	Milligram
ng	Nanogram
wt	Weight
m^2	Square metres

Dosing

id	Intradermal
im	Intramuscular
inh	Inhalation
ip	Intraperitoneal
iv	Intravenous
po	Oral
sc	Subcutaneous
mg/kg bw/d	mg/kg bodyweight/day
a.i.	Active ingredient

TOXICOLOGY

1.1 Review of supplementary studies of the mammalian toxicology and metabolism/toxiocinetics

1.1.1 Introduction

In 2002, following closure of the period for public comment on the draft diazinon review findings report, the APVMA received additional studies from registrants on the toxicology of diazinon. The APVMA requested the Office of Chemical Safety (OCS) to assess these additional studies and review the public health findings which had been reported in 2002.

Studies submitted

The studies submitted for assessment were as follows:

- The first study (Beilstein, 1998) was a 30-d repeat dose study in 4 human subjects using daily doses of 0.03 mg/kg bw. This study was designed to establish the safety of a dose of 0.02 mg/kg/d chosen for a subsequent clinical study;
- The second study was the aforementioned clinical study. This study was performed as an acute, single ascending oral dose design in human subjects and was aimed at establishing a NOEL for plasma and RBC cholinesterase activity. The study comprised three parts: a clinical phase (Part A: Boyeson, 2000); analysis of the metabolite diethylthiophosphate (DETP) in urine (Part B: Hughs & Vaughn, 2000); and, analysis of diazinon in blood and the diazinon metabolite (G-27550) in urine (Part C: Wong & Anderson);
- The third study was a dislodgeable residue study in which diazinon (and chlorpyrifos) were assessed for potential human exposure following application to residential and public turf areas (Merricks, 1987); this study was not considered suitable for public health risk assessment, because of the limited nature of the data and the fact that the exposure estimates were unreliable.

Current public health recommendations for diazinon

Acceptable Daily Intake (ADI)

The TGA's recent toxicological evaluation of diazinon (July, 1999) did not recommend a change to the then existing Australian ADI of 0.001 mg/kg bw/day, but changed the critical study on which it was set. The ADI was originally derived from a NOEL of 0.1 mg/kg bw/day for plasma ChE inhibition in a 3-month rat study.

The ADI was considered out of session following the 17th meeting of the ACPH held in April, 1999. The ACPH considered the assessment of a 37-43 day human study (Lazanas et al, 1966), with a lower NOEL (0.02 mg/kg bw/day) and based on the same endpoint, plasma cholinesterase. The ACPH recommended that the ADI be based on the lower NOEL of 0.02 mg/kg bw/day and that some consideration be given to including an additional safety factor to take into account the limited nature of that human study. The ACPH noted that plasma ChE inhibition appears to be the most sensitive toxicological endpoint for diazinon and that data from feeding studies (in excess of 3-months duration) indicates that no LOEL from any of the animal studies is much less than 0.02 mg/kg bw/day, the NOEL observed for humans in the 37-43 day repeat-dose study. Most LOELs for plasma ChE inhibition by diazinon occur between 0.01 and 0.025 mg/kg bw/day and the Committee agreed that using a "weight of

evidence" approach, 0.02 mg/kg bw/day is the threshold level for plasma ChE inhibition. The Committee supported the retention of the existing ADI for diazinon of 0.001 mg/kg bw/day on the basis of plasma ChE depression in humans at 0.02 mg/kg bw/d and the application of a 20-fold safety factor. The additional 2-fold safety factor was applied due to the closeness of the NOEL and LOEL (0.025 mg/kg bw/day) and the limited nature of the study.

Acute Reference Dose (ARfD)

The acute reference dose (ARfD) is an estimate of the amount of a chemical (residue) in food or water that can be ingested over a short period of time, usually during a meal or in one day, without an appreciable health risk. At the time the ARfD was set there were three human studies in the toxicology database for consideration. The current Australian ARfD of 0.005 mg/kg bw was based on the NOEL of 0.05 mg/kg bw/d for RBC ChE inhibition in the 5-day human study of Sze and Calandra (1965). A safety factor of 10 was used to derive the ARfD value.

Summary of studies in current submission

Diazinon in epoxidised soybean oil was administered po to clinically normal, healthy, adult male subjects between the ages of 18 and 48 as a single dose at 0, 0.03, 0.12, 0.20, 0.21 or 0.30 mg/kg bw (to 11, 7, 7, 7, 8 & 1 subjects, respectively) via a gelatine capsule. There was a dose-related, toxicologically and statistically significant plasma ChE inhibition commencing at about 4 h after dosing (32-78%) at and above 0.12 mg/kg bw relative to baseline values. At 0.3 mg/kg bw, time-related plasma ChE inhibition was observed relative to placebo controls (2-93%). The maximal inhibition was seen about 6 h after dosing (42-93%). Enzyme activity showed recovery with time, commencing at approximately 8 h post treatment. However, 7 individuals had still not recovered to below 20% plasma ChE inhibition by study completion (ie at 15 days after treatment). With respect to RBC ChE inhibition, the effects at 0.2 mg/kg bw were not statistically significant compared to placebo controls. At 0.21 mg/kg bw, the inhibition was slightly greater than that seen at 0.2 mg/kg bw and statistically significant (4-11% inhibition) relative to placebo controls. Inhibition at 0.21 mg/kg bw persisted from day one until the study completion on day 15 after dosing. Therefore, the dose level of 0.21 mg/kg bw may be considered as a LOEL for RBC ChE inhibition. At 0.3 mg/kg bw, the level of RBC inhibition ranged from 4-13% relative to placebo controls. The NOEL for plasma ChE inhibition was 0.03 mg/kg bw. Because there was a statistically significant inhibition of RBC ChE activity at 0.21 mg/kg bw, the NOEL for RBC ChE inhibition was 0.2 mg/kg bw. Since a justification for the selection of doses at 0.2 and 0.21 mg/kg bw was not provided, assigning a NOEL at 0.2 mg/kg bw should be cautiously interpreted (Boyeson, 2000).

Urine and plasma samples from the same subjects were analysed for diazinon and one possible metabolite (G-25770). Diazinon in plasma was detected in only some subjects at 0.12, 0.20 and 0.21 mg/kg bw. The plasma levels ranged from 1.3-3.0 ppb at 1, 2 and 4 h after dosing. The single subject given diazinon at 0.3 mg/kg bw had a plasma level of 5.9 ppb at about 4 h after treatment, which then decreased to 1.4 ppb at 6 h post dosing. Plasma levels appeared to have reached maximal concentrations at about 4 h after dosing. The average amounts of the metabolite, G-25770 [6-methyl-2-(1-methylethyl)-4-(1H)-pyrimidinone] excreted in the urine during the first 48 h of dosing represented about 8-25% of the administered diazinon dose, and generally, the amount excreted related to the diazinon dose administered. The majority of the metabolite found in urine was excreted within the first 48 h after dosing, with the rate of G-27550 excretion being faster during the first 24 h (Wong & Anderson, 2000).

Urine samples from the same subjects were also analysed for the presence of another major metabolite, diethylthiophosphate (DETP). Raw data were reported but no analysis was presented in this report and hence these data have not been evaluated (Hughes & Vaughn, 2000).

In another human study, diazinon technical in gelatine capsules was administered to four healthy male subjects at 0.03 mg/kg bw/d, once daily for 28 to 31 days. Treatment resulted in 22-48% inhibition in plasma ChE activity. The enzyme activity showed some signs of recovery at 31 days after cessation of treatment. Some fluctuations in RBC ChE activity were noted during the study, but these were in agreement with literature values for normal individual variations. All other tested study parameters were unaffected by treatment. However, the regulatory value of the study findings is limited as only four males were tested. The NOEL for plasma cholinesterase activity was <0.03 mg/kg bw/d. The single dose tested, 0.03 mg/kg bw, can be considered a NOEL for RBC cholinesterase activity (Beilstein, 1998).

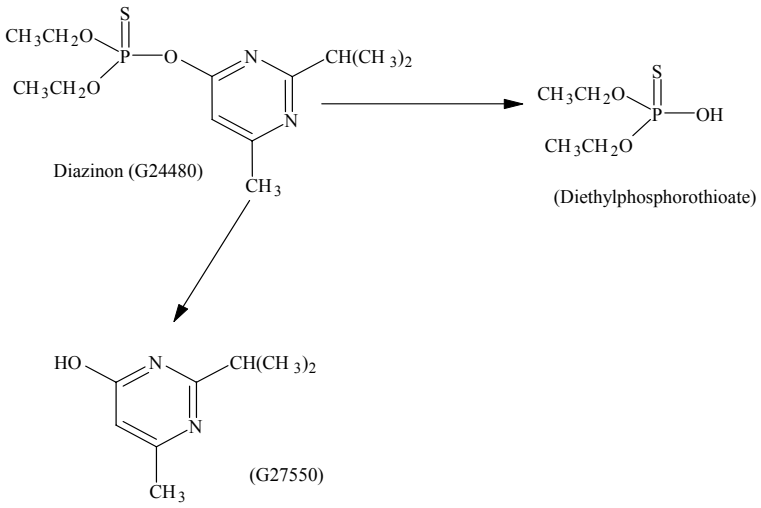
DISCUSSION

General toxicological information arising from the (E)CRP review

The toxicology database of diazinon is extensive and a large number of toxicology studies were submitted and evaluated for the initial CRP review. The supplementary studies evaluated in this report provide additional useful information.

The toxicological profile of diazinon is typical of most organophosphorus ChE-inhibiting pesticides, with clinical symptoms being similar in experimental animals and humans. The acute toxicity profile is characterised by rapid absorption and distribution, extensive metabolism and fast excretion in the urine. Stabilised diazinon (eg. diazinon in epoxidised soybean oil) is of moderate acute oral toxicity, with relatively large species differences in sensitivity. The CRP review concluded that female animals in some species (rats, dogs) tend to be more sensitive to diazinon-induced ChE inhibition, but this finding is not consistent. Signs of acute toxicity (oral, dermal, inhalation, intraperitoneal) were those typically seen in organophosphate intoxication and included muscarinic effects (diarrhoea, salivation, pupil constriction), nicotinic effects (muscle fasciculations and fatigue) and CNS effects (ataxia, convulsions). Technical diazinon was a slight eye and skin irritant and a skin sensitiser.

Following oral administration to rats, diazinon is almost completely absorbed from the GI tract and has plasma half-life of 2.9 h, consistent with rapid elimination from the circulation. Excretion studies in rats indicate that most of the absorbed diazinon (96-97%) is present in urine as metabolites within 24 h. The major metabolic pathway includes the oxidase/hydrolase-mediated cleavage of the ester bond leading to the low toxicity derivatives G-25770, [6-methyl-2-(1-methylethyl)-4-(1H)-pyrimidinone] and diethylthiophosphate (DETP) as shown below.



Human Toxicity & Toxicokinetics

Apart from characteristic clinical signs associated with acute cholinergic crisis following accidental or deliberate ingestion, diazinon may induce an additional paralytic condition called "Intermediate Syndrome" consisting of a sequence of neurological signs that develop some 24-96 h after poisoning (Samal & Sahu, 1990). This condition appears to develop before the onset of delayed neuropathy (so-called "organophosphate-induced delayed neurotoxocity" or OPIDN). Clinical signs of the "Intermediate Syndrome" can be distnigushed from the characteristic muscarinic, nicotinic and central nervous system (CNS) effects observed very soon after exposure as a delayed onset of muscular weakness affecting neck, proximal limb and respiratory muscles.

There have been no reported cases of OPIDN in humans following accidental or deliberate diazinon poisoning, a result consistent with the negative findings observed in animal studies.

The synopses of the human toxicity studies evaluated by the TGA in the recent (E)CRP review of diazinon are given below.

- A study, intended to monitor the effects of progressively increasing the oral dosage of diazinon in 3 subjects from 0.05 to 5.0 mg/kg bw/day over 13 weeks, was terminated after only 5 days when significant ChE inhibition in plasma (mean, 38%) was detected at the lowest dose of 0.05 mg/kg bw/day. After a 23-day recovery period, dosing at 0.05 mg/kg bw/day for 5 days was repeated and the extent of the significant plasma ChE inhibition observed in the first exposure confirmed (ie. 33%). There were no clinical signs or changes in bodyweight during treatment or recovery. Similarly, there was no skin sensitisation or any changes to any of the measured haematological (ie. Hb, erythrocyte count, total and differential leucocyte count and prothrombin time), clinical chemical (ie. BUN, AP and AST) or urinary parameters (ie. albumin, protein and microscopic elements) other than for plasma ChE activity. Significant plasma ChE inhibition ($p<0.05$) persisted for 6 days after treatment in the first cycle, and substantial though non-significant inhibition (ie. 22%) was observed for 6 days after treatment in the second. No changes in RBC ChE activity were observed during treatment. Therefore, based on significant inhibition of plasma ChE activity after a 5-day treatment at 0.05 mg/kg bw/day, a NOEL could not be established (Sze & Calandra, 1965).

- A daily oral administration of 0.0245-0.03 mg/kg bw/day diazinon for 33-34 days as a divided dose resulted in no clinical signs or changes in bodyweight, haematology or urinalysis in 4 subjects. However, although ChE activity in erythrocytes was apparently unaffected by the 34- or 36-day treatment, ChE activity in plasma in 2 subjects would appear to have been completely inhibited after a single administration, albeit from a pre-test activity of 5% and 13% respectively. A second administration after a 5-day recovery resulted in activities greater than pre-test levels with no apparent reduction by subsequent daily administration. Based on the observation that the inter-assay ChE variability was quite marked whereas intra-assay appeared relatively consistent, a comparison of results derived on the same day of assay suggests that significant inhibition (~40%) of ChE in plasma occurs. Thus, in the presence of a 60% reduction in acid phosphatase activity and 40% reduction in plasma ChE activity, a NOEL could not be established because only one dose was tested in this study (Payot, 1966).

{tc \l3 "***Ciba-Geigy Corp., Ardsley, New York, USA. Study duration: not stated. Report date: 22 Nov, 1966. (Validated and Pre-GLP)*** **}**

- Diazinon administered to groups of 3 adult male subjects in capsules for 37 days at 0.020 mg/kg bw/day or 43 days at 0.025 mg/kg bw/day caused no clinical signs or significant changes in bodyweight, haematology (ie. Hb, erythrocyte count, total and differential leucocyte count and prothrombin time), urinalysis (ie. pH and microscopic elements) or clinical chemistry (ie. BUN, AP and ALT) parameters except for plasma ChE activity. Treatment at 0.020 mg/kg bw/day resulted in a non-significant inhibition of ChE in plasma (ie. 8%) and erythrocytes. However, at 0.025 mg/kg bw/day a significant change in the mean plasma ChE inhibition relative to the combined mean pretest values for the treated subjects indicated a clear treatment-attributable effect. By contrast, mean erythrocyte ChE activity was not significantly inhibited at any time during treatment at 0.025 mg/kg bw/day. Recovery of plasma ChE activity was evident after cessation of treatment so that full activity returned by approximately day 61. Based on the significant inhibition of plasma ChE activity at 0.025 mg/kg bw/day, the NOEL for this study was established at 0.020 mg/kg bw/day (Lazanas et al, 1966)

In the human study of Wong & Anderson (2000) the plasma diazinon levels were between 1.0-3.0 ppb, except for the level of 5.9 ppb found in the single subject treated with diazinon at 0.3 mg/kg bw. These levels are consistent with the relatively low doses used in the study and are roughly proportional to the plasma levels found in studies in rats and mice. In mice treated with diazinon ip at 20 and 100 mg/kg bw, the blood diazinon concentrations at T_{max} were 100 and 750 ppb, respectively (Tomokuni et al, 1985). Similar kinetics have been observed in rats following ip administration at the same dose levels. About 8 h after dosing at 100 mg/kg bw, the blood diazinon level in rats was found to be 165 ppb (Tomokuni & Hasegawa, 1985).

The low amounts of radioactivity associated with unchanged diazinon found in the urine and faeces in rats, dogs, sheep and goats indicate that diazinon is extensively metabolised in these species (Mucke et al, 1970). After oral dosing, diazinon and its metabolites were mainly excreted in the urine in rats, and accounted for about 95-96% of the administered dose (Capps, 1989).

Although the human studies evaluated in this report did not quantify unchanged diazinon in the urine, the urinary excretion data for diazinon metabolite, G-27550 reported herein (Wong

& Anderson, 2000) is consistent with the findings in laboratory animals with respect to urinary metabolites. The data for DETP levels in urine presented in the report by Hughes & Vaughn, (2000), were not assessable.

The human study by Boyeson (2000) evaluated in this review recorded a NOEL of 0.03 mg/kg bw for plasma ChE inhibition following oral administration of a single dose, which is in agreement with the findings of Sze & Calandra (1965), who reported a NOEL of <0.05 mg/kg bw/d for plasma ChE inhibition in a 5-day repeat dose human study. However, it was slightly higher than the NOEL of <0.0245 mg/kg bw/d reported by Payot (1966) in a 33-34 day human study, and that of 0.02 mg/kg bw/d reported by Lazanas et al (1966) in a 37-43 day oral toxicity study for plasma ChE inhibition in humans. Given that the Boyeson (2000) study was a single dose study, this new finding would not warrant any changes to the current Australian ADI, which has been derived on the basis of the NOEL for plasma ChE inhibition in the human study of Lazanas et al (1966). However, this new study enabled identification of a new NOEL for RBC ChE inhibition in humans following po administration of a single dose, ie. 0.2 mg/kg bw. Based on this finding, it is possible to revise the Australian ARfD value for diazinon. Given the limited nature of this study (dose selection) and the closeness of the NOEL and LOEL, it is considered appropriate to include an extra 2-fold safety factor. Hence, application of a total 20-fold safety factor to the NOEL of 0.2 mg/kg bw would yield a new Australian ARfD value of 0.01 mg/kg bw.

The single dose human study of Beilstein (1998) evaluated in this report was conducted to ascertain the initial dose of the acute ascending-dose oral toxicity study of Boyeson (2000). A NOEL of <0.03 mg/kg bw for plasma ChE inhibition was recorded following oral administration of diazinon at 0.03 mg/kg bw/d for 28-31 days.

Conclusions

- Following the recent (E)CRP review of diazinon, no changes were recommended to the existing Australian ADI value of 0.001 mg/kg bw/day. However, the basis for this ADI was changed, and it was derived by applying a safety factor of 20 to the NOEL of 0.02 mg/kg bw/day in a 37-43-day human study by Lazanas et al (1966) rather than on the basis of a NOEL of 0.1 mg/kg bw/d for plasma ChE inhibition in a 3-month rat study. The additional 2-fold safety factor was applied because of the closeness of the NOEL and LOEL in this study, uncertainty surrounding the impurity profile of the administered diazinon (ie. TEPPs content) and the limited nature of the study (two doses and 3 subjects/group). The present review has not identified a suitable study that would warrant revision of this ADI.

- In 1999 an acute reference dose (ARfD) of 0.005 mg/kg bw was established for diazinon, based on a NOEL of 0.05 mg/kg bw for RBC ChE inhibition in a 5-day human study (Sze & Calandra, 1965) and applying a 10-fold safety factor. The present review has evaluated a recent single-dose human oral toxicity study (Boyeson, 2000), which reported a NOEL of 0.2 mg/kg bw for RBC ChE inhibition based on significant inhibition at the next highest dose of 0.21 mg/kg bw. As this is an acute oral dose study, it is the most suitable study available for derivation of the ARfD. It is recommended that the ARfD be set at 0.01 mg/kg bw, based on the NOEL of 0.2 mg/kg bw and using a 20-fold safety factor. The additional 2-fold safety factor over the default 10x is applied because of the limited nature of the study (dose selection) and the closeness of the NOEL and LOEL.

1.1.2 Main Toxicology Report

Metabolism and toxicokinetics

Wong AJ & Anderson GD (2000) A randomized, double-blind, ascending, acute, oral dose study of diazinon to determine the No Effect Level (NOEL) for plasma and RBC cholinesterase activity in normal, healthy subjects. Part C: Analysis of diazinon in blood and G-27550 in urine. Study No. NCP-8373-Part C. Lab: Development Resources/Chemical Support Department, Novartis Crop Protection Inc. Sponsor: Novartis Crop Protection Inc. Study duration: Clinical trial was conducted during 26 July-22 October, 1998. Report No. Novartis No: 615-98, Report date: July 25, 2000.

[Report contains the data from the analytical portion study of Boyeson MG (2000); Study No. NCP-8373 Part A; blood diazinon levels: summaries plus individual data].

This report presented the results of analysis of blood diazinon and the urinary diazinon metabolite (G-25770 [6-methyl-2-(1-methylethyl)-4-(1H)-Pyrimidinone,] from the acute human study by Boyeson (2000). In that main study (clinical phase), 41 clinically normal, healthy, adult male subjects between the ages of 18 and 48 received a single oral dose of diazinon (purity: 8% w/v, in epoxidized soybean oil, batch: 781/44, source: Novartis Crop Protection) at 0.03, 0.12, 0.20, 0.21 or 0.30 mg/kg bw administered po via a gelatine capsule with 240 mL of water after consuming a standardised breakfast. There were 7 subjects/group at 0.03, 0.12, 0.20, 0.21 mg/kg bw, whilst the 0.3 mg/kg bw group consisted of only one subject. The placebo control group consisted of 11 subjects (see below for full experimental details). Blood samples for the plasma diazinon determinations were collected at check-in (2 days before) and just prior to dosing on day 0 (0 h), 1, 2, 4, 6, 8, 12, 24 and 48 h, and 5, 8 and 15 days post dosing. Plasma diazinon and urinary diazinon metabolite, G-27550 were analysed by GC/MSD methods developed by the study performing laboratory. It was reported that the method used for plasma diazinon assay was a method validated under GLP, whilst that used for urinary metabolite determination was validated during the course of the study.

Table of subject identification numbers and treatment

Sample	***Dose (mg/kg bw)***					
	Control	***0.03***	***0.12***	***0.2***	***0.21***	***0.3***
Plasma	*2, 6, 11, 14, 20, 21, 24, 26, 31, 34, 36, 38*	*1, 3, 4, 5, 7, 8, 9*	*10, 12, 15, 16, 17, 18, 19*	*32, 33, 35, 37, 39, 40, 41*	*13, 22, 23, 25, 27, 28, 30*	*29*
Urine	*2, 6, 11, 14, 20, 21, 24, 26, 31, 34, 36, 38*	*1, 3, 4, 5, 7, 8, 9*	*10, 12, 15, 16, 17, 18, 19*	*32, 33, 35, 37, 39, 40, 41*	*13, 22, 23, 25, 27, 28, 30*	*29*

Protocol deviations: *[See evaluation report of the study of Boyeson (2000) for the other protocol deviations.]*

Blood collection/processing: Blood sample were collected 1-28 minute later than originally planned; sometimes the samples were not centrifuged within 30 minutes of collection, but were 1-16 minutes late.

Urine collection/processing: The 6-12 h urine sample for subject 20 (control) and the 12-24 h sample for subject 33 (at 0.2 mg/kg bw) were not collected, and the 6-12 h, day 4 and day 14 urine samples for subject 21 (control) were not collected.

The samples in the preliminary recovery studies were corrected for control residues. Test samples from the clinical phase were not corrected for control residues. However, they were corrected for the procedural recovery values (if recovery was <100%). Descriptive statistics were available.

Findings:
The limit of quantification (LOQ) of the plasma diazinon assay was 1.0 ppb. The urinary G-27550 assay had a LOQ of 1.0 ppb during the course of the study. Average recoveries of 70-100% were considered acceptable. The magnitude and frequency of outliers from this recovery range were evaluated as needed. In this study, the overall average recovery of diazinon from fortified plasma samples (from 1.3 to 100 ppb) was 104% (range 58-134%; SD 13%), whilst that for the urinary metabolite, G-27550, was 94% (range: 50-160%; SD 19%). Preliminary studies on the stability of diazinon in plasma and G-27550 in urine showed that these compounds are stable for at least 18 and 12 months in frozen samples, respectively, representing 96% and 104% of the fortified amounts at these two time points.

Plasma levels: Diazinon was not detected in plasma of the control or treated subjects given diazinon at 0.03 mg/kg bw. Some samples collected from the 0.12 (1 subject), 0.20 (2 subjects) and 0.21 (1 subject) mg/kg bw groups contained quantifiable amounts of diazinon ranging from 1.3-3.0 ppb at 1, 2 and 4 h after dosing, but not thereafter (see Table below). All other plasma samples collected at different sampling times from these three dose groups contained <1.3 ppb diazinon. The single subject treated at the 0.3 mg/kg bw level exhibited a plasma diazinon level of 5.9 ppb at about 4 h after treatment, which decreased to 1.4 ppb by 6 h post dosing. The plasma diazinon levels appeared to have reached maximal concentrations at about 4 h after dosing.

Plasma diazinon levels (ppb) in four subjects at different sampling times

Dose (mg/kg bw)	*Subject Number*	*Time after dosing (h)*					
		0	*1*	*2*	*4*	*6*	*8*
0.12	***15***	***<1.3***	***2.8***	***<1.3***	***<1.3***	***<1.3***	***<1.3***
0.20	***32***		***1.9***	***<1.3***	***3.0***		
	37		***<1.3***	***2.5***	***<1.3***		
0.21	***23***		***2.0***	***<1.3***	***<1.3***		
0.30	***29***		***<1.3***	***<1.3***	***5.9***	***1.4***	***<1.3***

Urinary excretion: Percentages of the administered dose excreted as G-27550 in urine by the subjects for different sampling periods during the 48-h period after treatment are summarised in the Table below. The average amounts of G-25770 excreted in the urine during the first 48 h of dosing represented about 8-25% of the administered dose. Generally, the amount excreted was related to the administered diazinon dose, except at 0.20 mg/kg bw. The rate of excretion appeared to be faster during the first 24 h after dosing.

Urinary DETP levels: Only individual data for urinary DETP levels were provided and no statistical analyses were conducted on the data. According to the data, generally, the excretion of DETP in the urine was related to the dose administered.

*Percentage of the dose excreted in the urine as G-27550**

Dose (mg/kg bw)	***Body weight (kg)***	***Sampling interval (h)***				***Total (%)***
		0-6	*6-12*	*12-24*	*24-48*	
0.03	*82.1*	*2.5*	*2.0*	*2.2*	*1.13*	*7.9*
0.12	*76.1*	*2.9*	*3.3*	*3.4*	*1.7*	*11.3*
0.20	*78.6*	*2.7*	*2.0*	*2.5*	*1.1*	*8.1*
0.21	*75.4*	*2.2*	*8.3*	*1.95*	*0.88*	*13.4*
0.3	*71.2*	*1.8*	*17.0*	*3.3*	*3.2*	*25.3*

**Mean values calculated by reviewing toxicologist.*

Conclusions: Diazinon was detected in plasma of some subjects after dosing at 0.12, 0.20 and 0.21 mg/kg bw. Plasma levels ranged from 1.3-3.0 ppb and were present up to 4 h after dosing. The single subject given diazinon at 0.3 mg/kg bw had a plasma level of 5.9 ppb at about 4 h after treatment, which then decreased. Plasma levels appeared to have reached maximal concentrations at about 4 h after dosing. The average amounts of the urinary metabolite, G-25770 excreted in the urine during the first 48 h of dosing represented about 8-25% of the administered diazinon dose. The proportions of the dose excreted in the urine as G-27550 generally increased as the dose increased (at least over the range 0.03 –3.0 mg/kg bw). The rate of G-27550 excretion was faster during the first 24 h after dosing.

Hughes DL & Vaughn C (2000) A randomized, double-blind, ascending, acute, oral dose study of diazinon to determine the No Effect Level (NOEL) for plasma and RBC cholinesterase activity in normal, healthy subjects. Part B: Analysis of DETP in urine. Study No. NCP-8373-Part B, 6117-394. Lab: Covance Clinical Research Unit Inc. Sponsor: Novartis Crop Protection Inc. Study duration: Clinical trial was conducted during 26 July-22 October, 1998. Report No. Novartis No: 587-98, Report date: July 25, 2000.

[Report contained the urinary DETP data (individual) of the study of Boyeson MG (2000) (Study No. NCP-8373 Part A)].

Only raw data were provided in this report and it was considered to be not assessable. However, in general, the proportions of DETP excreted in the urine were related to the administered diazinon dose.

Acute toxicity

Boyeson MG (2000) A randomized, double-blind, ascending, acute, oral dose study of diazinon to determine the No Effect Level (NOEL) for plasma and RBC cholinesterase activity in normal, healthy subjects. Study No. NCP-8373 Part A. Lab: Covance Clinical Research Unit Inc. Sponsor: Novartis Crop Protection Inc. Study duration: Clinical trial was conducted during 26 July-22 October, 1998. Report No. Novartis No: 587-98, Report date: July 25, 2000.

Quality assured study conducted in compliance with the US Code of Federal Regulations governing the protection of human subjects (21 CFR 50), Institutional Review Boards (21 CFR 56) and the Obligations of Clinical investigators (21 CFR 312), which are consistent with the Declaration of Helsinki and Common Rule.

Study

Forty clinically normal, healthy, adult male subjects between the ages of 18 and 48 (bw range 64.3 – 99.5 kg; mean: 78.9 kg) participated in the study. These subjects received a single oral dose of diazinon (purity: 8% w/v, in epoxidized soybean oil, batch: 781/44, source: Novartis Crop Protection) at 0.03, 0.12, 0.20, 0.21 (7 subjects/group) or 0.30 (1 subject) mg/kg bw administered po via a gelatine capsule with 240 mL of water after consuming a standardised breakfast. The placebo control group consisted of 11 subjects. The study utilised a double-blind, randomised, ascending dose design. A single individual (a dose cohort leader) in a dose-block received the next highest dose, while the remainder of the group received a lower dose that had been demonstrated to be safe and tolerable by the previous dose cohort leader (see Table below for dosing regimen).

Dose preparation and dosing

Each dose (in a gelatine capsule) was prepared within 24 h prior to dosing, stored under refrigeration, and protected from light until use. For each dose block, one extra capsule each of diazinon and placebo vehicle were prepared. These were subsequently analysed for diazinon.

Subjects were instructed to swallow the capsule whole. The subjects entered the study 2 days prior to dosing and abstained from consuming foods or beverages that contained xanthine or caffeine for 5 days, and that contained alcohol for 14 days prior to study entry up until study completion on day 15. They also refrained from consuming grapefruit/juice during the study. They were provided with standardised diets (breakfast, lunch, dinner and a snack) moderate in fibre with normal fat levels. Consumption of water was *ad libitum* throughout the study. The subjects also refrained from strenuous exercise during the study and from the use of prescription or non-prescription medications within 14 days prior to study entry and for the study duration.

Although it was originally planned to include females in the study after completion of male cohorts, this was not done on the advice of the sponsor. The initial dose of 0.03 mg/kg bw was based on the findings of a single dose, 28-31 day repeat exposure study in human subjects (Beilstein, 1998). It was reported that in animal studies, rats have been shown to tolerate, without effect, a single acute oral dose that is approximately 10 x the NOEL established in repeat-dose studies. Based on this relationship observed in rats, it was anticipated that humans may tolerate a dose of 0.3 mg/kg bw diazinon without effect. Dose levels were incremented based on the criteria outlined below.

All subjects were pre-screened within 4 weeks prior to study commencement for recording of complete medical history, physical condition, ECG, blood pressure, pulse rate, clinical chemistry tests, haematology, urinalysis, serology for hepatitis B and C, and plasma and RBC ChE assays. Only those subjects who met the following criteria were included in the study: good health, normal resting blood pressure and heart rate, body weight between 50 and 100 kg, negative HIV and hepatitis B & C results and negative urine test results for drugs of abuse at screening and on day –2 of the study. Factors that excluded potential subjects from the study were the following: any acute or chronic conditions, history or clinical manifestations of significant metabolic, pulmonary, cardiovascular, hepatic or renal disease or condition, alcoholism within a year of study entry, allergic conditions, donation or loss of >400 mL blood within 12 weeks prior to study entry, and participation in other clinical studies ending within 90 days of study entry.

Dosing regimen

Dose block	Dose (mg/kg bw)	Number of subjects
1	0	1
	0.03	1*
2	0	2
	0.03	6
	0.12	1*
3	0	3
	0.12	6
	0.21	1*
4	0	3
	0.21	6
	0.3	1*
5	0	3
	0.20	7

*Lead subject for the dose group.

The decision to proceed to the next dose block was based on the following criteria:

- Absence of clinically significant adverse events indicative of test material intolerance. If the lead-in subject of a dose level/dose cohort group or if two or more subjects in any one dose level developed a severe, probably-related adverse experience, or if one subject developed a serious, probably-related event that was life-threatening, then that dose level was considered an intolerable dose.
- Clinically significant physical examination, ECG findings or vital signs in two or more subjects at any dose level
- An overall pattern of clinical changes or symptoms which could have appeared minor in terms of individual adverse events but which collectively represented a concern of safety
- Levels of RBC ChE inhibition >20% from baseline, if concomitant with plasma ChE inhibition >20% of baseline (each subject's baseline ChE level was defined as the level just prior to dosing). If a single individual at any dose level reached >20% inhibition of RBC ChE (concomitant with >20% inhibition of plasma ChE inhibition), no further increase in dose level was attempted. However, if this occurred, the next cohort group dose level could have been adjusted downward in order to help define the upper limit of NOEL and,
- Presence of a pattern of clinical laboratory changes ie, consistent increase or decrease within a dose level or within a randomised dose group, which might have indicated an overall safety of concern.

Check-in procedures (day –2)

Subjects reporting to the clinical facility were subjected to medical and clinical chemistry examinations as outlined above. Blood samples were taken for assay of diazinon and urine for assay of diazinon metabolite G-277550 (see below for details).

Observations

Serial: Blood samples for haematology and clinical chemistry evaluations were collected (at and 24 and 48 h post dose, and study completion on day 15) via direct venipuncture or in-dwelling catheter. The following haematological variables were determined: RBC, WBC, WBC-DC, Hct, Hb, MCH, MCHC, MCV and MPV (mean platelet volume). The tested clinical chemistry parameters included the following: albumin, AP, ALT, AST, BUN,

amylase, calcium, chloride, cholesterol, CPK, creatinine, GGT, glucose iron, LDH, lipase, phosphorous, potassium, sodium, total bilirubin, total protein, CO2, triglycerides and uric acid. Plasma and RBC ChE activities were measured using blood samples collected at the following time points: prior to dosing on day 0, and at 1, 2, 4, 6, 8, 12, 24 and 48 h post dosing. The enzyme activities were determined according to the method of Ellman et al (1961), with thiocholine as the substrate. Urinalysis (pH, specific gravity, urobilinogen and creatinine) was conducted at 1, 2 and 16 days post dosing.

Plasma diazinon & urinary metabolite analyses

Experimental methods and the findings are described elsewhere in this report (Wong and Anderson, 2000, and Hughes and Vaughn, 2000).

Descriptive statistics were calculated for the following parameters: subject demographics, incidence of adverse effects, clinical chemistry and haematology data, urinalysis data, vital signs and ECG findings. Where appropriate, the following tests were used to analyse the data: one-way ANOVA, Dunnett's and paired t-test.

Blood samples collected at screening, check-in on day –2 and day –1 were analysed on separate occasions and represented the day-to-day pre-dose variations for each individual. This pre-dose variation for each subject was used to determine whether the subject had recovered following dose administration (eg. reached discharge criteria on day 15 post dosing). Data collected from Group 1 samples at days –10, -7 and –4 were considered as informational data on baseline variability, and were not included in any analyses. The samples collected at 0, 1, 2, 4, 6, 8, 12 and 24 h were analysed at the same time. The 0 h plasma and RBC ChE values were regarded as the baseline data for these two variables.

Protocol deviations

The following protocol deviations for various aspects of the study were reported.

Inclusion/exclusion: Two subjects (subjects no. 12 & 18) were not fasted prior to screening and two other subjects (subjects no. 17 & 32) were overweight according to the 1996 Metropolitan Height and Weight Tables; caffeine/xanthine test was not performed on one subject (subject no. unspecified); subject no. 37 had allergies to pollen and dust that required medication; and subject no. 28 took chewable antacid 13 days prior to study entry.

Check-in: Subject No 21 (placebo control) voluntarily withdrew from the study for personal reasons and exited on day 8 of the study; two subjects did not fast for clinical laboratory tests that were conducted on days –7 and –4, respectively; and two subjects (3 & 11) did not meet the criteria for ranges of vital signs.

Vital signs: Subjects were not always seated for at least 5 minutes prior to vital signs were taken; the time of the day vital signs were measured for subject 30 was unknown, and his blood pressure at 48 h post dosing was unknown.

Findings

All subjects enrolled in the study were considered healthy as they met all the defined inclusion criteria and, did not meet any of the exclusion criteria. A total of 42 adverse events were recorded. Forty-one of the 42 events showed no association with treatment (eg. accidental injury, asthenia, chills, headache, neck pain, diarrhoea, flatulence, nausea, vomiting, ecchymosis, lymphadenopathy, cough, pharyngitis, rhinitis, rash, skin

discolouration, ear pain). All of these events occurred either pre-dose or in subjects receiving placebo control capsules, and were classified as "mild" in severity. Back pain was reported at 7 days post dosing in one subject given 0.3 mg/kg bw diazinon; this event was classified as "possibly related to treatment" by the study authors. The pain was reported to have resolved on the same day without any treatment. No further adverse events attributable to the test substance were reported.

No treatment-related abnormalities were detected in any individual on physical examinations, in vital signs or ECGs during the study. There were no treatment-related effects on any of the tested clinical laboratory parameters.

Plasma ChE activity

Dose-related and statistically significant ($p \leq 0.05$) plasma ChE inhibition was observed at and above 0.12 mg/kg bw (see Table below) at the majority of the observation times compared to placebo controls. For all dose groups, toxicologically significant enzyme inhibition was first reached at about 4 h post treatment; compared to placebo controls inhibition ranged from 32-78%. Maximal inhibition was recorded at about 6 h after the test substance administration (42-93% inhibition). A similar pattern of inhibition was seen if inhibition was calculated relative to baseline values rather than to placebo controls. No statistical tests were conducted for the single individual in the 0.3 mg/kg bw group. Nonetheless, plasma ChE inhibition at this dose level ranged from 2-93% in comparison to placebo controls, with the maximal inhibition of 93% occurring at about 6 h after dosing.

Plasma cholinesterase levels showed signs of recovery commencing at 8 h post treatment. This recovery was lengthy and 7 individuals (2 each at 0.12, 0.2 and .021 mg/kg bw and the single individual at 0.3 mg/kg bw) had not returned below 20% plasma ChE inhibition by study discharge (at 15 days post dosing).

Plasma ChE activity (µmol/L)[a] at different observation times

Dose (mg/kg bw)	**Observation time (hours or days after dosing)**					
	0 h (baseline value)	**1 h**	**2 h**	**4 h**	**6 h**	**8 h**
Control	4211± 610	4226 ± 676	4232 ± 634	4335 ± 631	4289 ± 593	4320 ± 581
0.03	4694 ± 681	4576 ± 675 (2.8%)	4612 ± 705	4456 ± 606 (4.8%)	4388 ± 586 (6.3%)	4389 ± 613 (6.3%)
0.12	4404 ± 1166	4105 ± 1384 (7.9%)	4046 ± 1392 (9.4%)	3049 ± 1292 (32.5%)	2524 ± 586 (41.6%)	2580 ± 625 (40.5%)
0.20	4210 ± 915	4209 ± 852	3688 ± 1087 (12.7%)	1942 ± 574 (52.8%)	1668 ± 262 (58.4%)	1713 ± 247 (57.3%)
0.21	4126 ± 472	3686 ± 581 (9.8%)	3470 ± 480 (14.7%)	1894 ± 561 (54.2%)	1574 ± 571 (62.2%)	1788 ± 614 (57.1%)
0.3[b]	3517	3516 (2%)	3406 (2%)	953 (78%)	268 (93%)	388 (91%)
Continued						
	12 h	**24 h**	**48 h**	**5 days**	**8 days**	**15 days**
Control	4233 ± 595	4317 ± 575	4236 ± 549	4062 ± 585	4148 ± 467	4121 ± 549
0.03	4398 ± 613 (6.3%)	4443 ± 562 (5.1%)	4479 ± 560 (4.2%)	4146 ± 619 (11.7%)	4421± 746 (6.0%)	4189 ± 632 (10.7%)
0.12	2580 ± 625 (38.9%)	2872 ± 666 (33.7%)	2859 ± 709 (34.5%)	3084 ± 823 (29.7%)	3511 ± 1040 (20.6%)	3722 ± 1019 (15.5%)
0.20	1713 ± 247 (55.4%)	2073 ± 201 (48.8%)	2200 ± 215 (46.0%)	2699 ± 281 (34.2%)	3096 ± 370 (24.8%)	3558 ± 460 (13.4%)

0.21	1788 ± 614 (57.4%)	2044 ± 537 (50.8%)	2388 ± 566 (42.5%)	2723 ± 571 (34.3%)	2978 ± 609 (28.2%)	3333 ± 500 (19.2%)
0.3[b]	453 (89%)	810 (81%)	1116 (73%)	1719 (57%)	2083 (50%)	2496 (39%)

[a]Mean ± SD; enzyme activity expressed as µmol/L = µmoles of thiocholine produced/min/L. Values in parentheses represent mean percent inhibition compared to placebo controls. [b]n = 1).

RBC ChE activity

The relevant data are presented in the following Table. Mean RBC ChE activity was not inhibited more than 13% relative to pre-dose base line value at any dose level.

- At 0.2 mg/kg bw, RBC ChE activity was inhibited by 8%, 3%, 1.5% at 1% at 2, 5, 8, and 15 days after dosing respectively compared to baseline values. When percent inhibition was calculated against the placebo controls, the 8% inhibition at 48 h became 2%, with no inhibition at the other observation times. The inhibition was not statistically significant by either method of calculation. Hence the effects seen at 0.2 mg/kg bw at 48 h after dosing while probably treatment-related are not considered to be of sufficient magnitude to be regarded as toxicologically significant.
- At 0.21 mg/kg bw, an analysis using an among group comparison of placebo *vs* all other treatment groups (one-way ANOVA and Dunnett P-value) revealed that RBC ChE inhibition was statistically significant in comparison to placebo controls at each of the 5, 8 and 15 d post-dose sample times. This inhibition was sustained at about 10% during this period.
- At 0.3 mg/kg bw, the level of RBC inhibition ranged from 4-13% relative to pre-dose baseline values. The single individual at 0.3 mg/kg bw showed an inconsistent pattern of RBC cholinesterase inhibition with the maximum inhibition (compared to baseline) of 13% at 15 d post dose. Additionally, enzyme inhibition was greater than that observed at 0.20 mg/kg bw and was generally persistent from day 1 post-dose onwards.

Although the group mean data at 0.21 mg/kg bw exhibited no marked inhibition, with statistical significance from day 5 after dosing onwards, individual data showed significant inhibition in one subject at 5 days (20.5%) and in another on three occasions (at 4 h and 5 and 8 d; 20-21% inhibition) after dosing relative to pre-dose baseline values. As the latter individual satisfied a criterion for cessation of dose escalation as defined in the study protocol, no additional dose escalations were performed and further subjects were dosed at the lower dose of 0.2 mg/kg bw. Additionally, these two individuals and another volunteer at 0.21 mg/kg bw showed >10% (the magnitude of inhibition at which the group mean values reached statistical significance compared to placebo controls; see Table below) RBC ChE inhibition compared to pre-dose baseline values, generally with >50% concomitant inhibition in plasma ChE activity on day 1. Based on these observations, the 0.21 mg/kg bw dose level was considered to be an effect level (LOEL) for RBC ChE inhibition. No subjects at 0.2 mg/kg bw showed significant inhibition in RBC ChE activity relative to pre-dose baseline values on day 1, albeit plasma ChE activity in this group was inhibited by about 50% compared to pre-dose baseline values.

RBC ChE activity (µmol/g) at different observation times[a]

Dose (mg/kg bw)	**Observation time (hours or days after dosing)**					
	0 h (baseline value)	**1 h**	**2 h**	**4 h**	**6 h**	**8 h**
Control	8426± 1039	8511 ± 1049	8426 ± 965	8489 ± 978	8532 ± 707	8435 ± 884
0.03	8439 ± 1237	8412 ± 1671	8412 ± 1397	8808 ± 1584	8449 ± 1385	8686 ± 1630

0.12	9698 ± 911	9756 ± 1101	9676 ± 947	9711 ± 802	9758 ± 1316	9494 ± 1021
0.20	8709 ± 612	8734 ± 750	9016 ± 754	9015 ± 607	8689 ± 521	8676 ± 707
0.21	8776 ± 763	8607 ± 901	8412 ± 658 (4%)	7883 ± 481 (10%)	8975 ± 908	8165 ± 742 (7%)
0.3[b]	9145	8552 (6%)	8517 (7%)	8795 (4%)	8691 (5%)	7976 (13%)
Continued						
	12 h	**24 h**	**48 h**	**5 days**	**8 days**	**15 days**
Control	8654 ± 1008	8538 ± 854	8236 ± 927	8282 ± 635	8550 ± 800	8481 ± 929
0.03	8614 ± 1233	8459 ± 1410	8566 ± 1258	8881 ± 1696	8574 ± 1547	9845 ± 1447
0.12	9928 ± 1104	9666 ± 1305	10242 ± 1687	9633 ± 1573	10304 ± 1746	10097 ± 1764
0.20	8726 ± 771	8743 ± 842	8043 ± 702 (8%)	8407 ± 894 (3%)	8574 ± 747 (1.5%)	8611 ± 815 (1%)
0.21	8853 ± 785	8840 ± 400	8275 ± 821 (5%)	7816 ± 907 (11%)*	7963 ± 787 (9%)*	7896 ± 526 (10%)*
0.3	9285	9756	8709 (5%)	8587 (6%)	8988	7924 (13%)

[a]Mean ± SD; *Significantly different from controls ($p \leq 0.05$, Dunnett's test). Values in parentheses represent mean percent inhibition compared to corresponding baseline values. [b]n = 1).

Conclusions: Based on the findings at and above 0.12 mg/kg bw, the NOEL for plasma ChE inhibition was 0.03 mg/kg bw. Because there was a statistically significant inhibition of RBC ChE activity in two individuals, the NOEL for RBC ChE inhibition was 0.2 mg/kg bw. Since a justification for the selection of doses at 0.2 and 0.21 mg/kg bw was not provided, assigning a NOEL at 0.2 mg/kg bw should be cautiously interpreted.

Short-term repeat dose studies

Beilstein P (1998) Tolerance study in Novartis managers upon repeated oral administration of diazinon. Study No. 972019, Lab: Novartis Crop Safety/Human Safety Assessment, CH-4002 Basel, Switzerland. Sponsor: Novartis Crop Protection AG, BU Insect Control, CH-4002, Basel, Switzerland. Study duration: October-December, 1997. Report No. 972019, Report date: March 13, 1998.

Non-quality assured study

Study: Diazinon (purity: 99.5%, batch: AMS 140/7, source: Swiss Caps, Switzerland) in gelatin capsules was administered to four healthy, male subjects at 0.03 mg/kg bw/d, once daily for 28 to 31 days. The dose level was apparently chosen based on an assessment of previous human studies and the purity of currently manufactured diazinon TGAC. It was expected that the dose level of 0.03 mg/kg bw/d would be a NOEL for the study. The capsules were analysed for the diazinon content before and after the dosing period by the study sponsor. Body weight of the subjects ranged from 79.5-95.0 kg (age: unspecified). Prior to commencement of the study, all subjects were screened for eligibility.

Screening included recording of personal data, medical history, 12-lead ECG, comprehensive physical examination (blood pressure, heart rate, heart rhythm, pulmonary system, lymph nodes, skin, abdomen including liver spleen, height, weight and oral temperature), neurological tests (motor and sensory reflexes, optical mobility, Romberg test, finger-nose test and Babinski test), haematology (RBC, Hb, Hct, PCV, MCHC, MCH, WBC and WBC-DC), clinical chemistry tests (glucose, C-reactive protein, ALT, γ-GT, AP, creatinine, uric acid and cholesterol), plasma and RBC ChE assay, urinalysis (protein, glucose, ketones, urobilinogen, bilirubin, pH and blood), and serological tests for HIV and hepatitis B. These tests were performed 28 days (subjects 1, 2 & 3) and 27 days (subject 4) days prior to commencement of the study. During the course of the study, an inhibition of RBC ChE

activity to 70% or below of pre-test values was considered as the criteria to discontinue or interrupt the test substance administration.

Observations

After completion of the screening, the baseline values for all of the above study parameters (except serology) were determined at d-27 (subjects 1 & 2) or d-7 (subjects 3 & 4) prior to initiation of dosing, and on d-1 (all subjects) of the study. On the day before the first dosing day, the subjects were clinically examined for any acute disorders or any changes that might have occurred since the screening and baseline determinations. The time point of the first administration of the test compound was designated as day 0.

Blood samples were collected for plasma and RBC assay at 6 h, and 1, 2 or 3, 8, 10 (only 3 subjects), 13 or 14 and 20 days during the treatment phase, and at 28, 29 or 30, and 48 or 57 days during the post treatment phase. Plasma ChE activity was assayed by the method of Ellman et al (1961), while RBC ChE activity was measured by the method of Augustinsson et al (1978). All other clinical assessments (as above) and urinalysis were performed at 8, 13 or 14, 20, 28, 29 or 30 days. The final examination, which included all clinical chemistry tests (except serology), assessment of neurological status and physical examination was conducted one day after the last intake of the test compound.

It was reported that 24-h urine samples were collected from one subject at 7 and 4 days prior to commencement of the study and then on study days 0, 1, 3, 7, 14 and 28 for optional kinetic and metabolic analyses. However, none of the study parameters related to these study areas were specified, and no statistical tests were conducted to analyse the data.

Findings:

Physical examination, neurological status & ECG: No abnormalities were detected at any of the observation points.

Clinical chemistry & haematology: Administration of diazinon had no effect on any of the clinical chemistry parameters tested. In haematology, marginally elevated leucocytes (12.4 K/µL; normal range: 3.0-10 K/µL) and segmented neutrophils (74%; normal range: 45-70%) were noted in one subject at 14 days during treatment. No toxicological significance was attributed to these isolated findings, given that they were not repeated, and were limited to one subject.

Plasma ChE activity: Individual values of the four subjects are presented in the Table below. In comparison to pre-test values, treatment-related and toxicologically significant plasma ChE activity inhibitions (22-48%) were observed in all four subjects from study day 8 onwards. The inhibition was seen to persist in all four subjects, showing signs of enzyme activity recovery by day 31 after cessation of treatment.

RBC ChE activity: Some fluctuations in RBC ChE activity (generally increases) were noted in all four subjects, with a mean coefficient of variation of 9.5% (range 6-13%), similar to the normal coefficient of variation of 10% reported in the literature (Jokanovic & Maksimovic, 1997).

Plasma ChE activity (U/L)[a]

Study day	Subject 1	Subject 2	Subject 3	Subject 4
Pre-test				
-28	8950	8894	11597	ND
-27	8740	8943	ND	10890
-7	ND	ND	11392	10918
-1	8386	7592	10792	9575
Mean	8692	8476	11262	10461
Treatment period				
0	8085 (7%)	7415 (13%)	10692 (5%)	9739 (7%)
1	8168 (6%)	7711 (9%)	10442 (7%)	10387 (1%)
2	8362 (4%)	8308 (2%)	ND	ND
3	ND	ND	10315 (8%)	9262 (11%)
8	5103 (41%)	6593 (22%)	7353 (35%)	6061 (42%)
13	4557 (52%)	ND	ND	ND
14	ND	5390 (36%)	5500 (51%)	5719 (45%)
20	4653 (46%)	4454 (47%)	5077 (55%)	5895 (44%)
Post-treatment				
28	ND	ND	5696 (49%)	5631 (46%)
29	ND	5052 (40%)	ND	ND
31	3958 (54%)	ND	ND	ND
48	7639 (12%)	ND	9953 (12%)	9579 (8%)
57	ND	7287 (14%)	ND	ND

[a]Values in parentheses represent percent inhibition. ND = not determined.

Conclusions: Oral administration of diazinon in gelatine capsules to human subjects at 0.03 mg/kg bw/d for 28- 31 days resulted in 22-48% inhibition in plasma ChE activity. The enzyme activity showed some signs of recovery by 4 weeks after cessation of treatment. Some fluctuations in RBC ChE activity were noted, but the observations were consistent with normal individual variations. It is not stated why no observations or cholinesterase assays were performed during the last 8 days of dosing. This study was clearly a dose-safety confirmation test for a subsequent clinical study and is considered to be of limited regulatory value, as only four male subjects were tested without reference to GLP standards.

1.1.3 References

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1.2 Preliminary consideration of inhalation exposure

1.2.1 Introduction

Diazinon is an organophosphorus insecticide which has been widely used in Australia for over 30 years. The chemical has been the subject of a major review under the APVMA's Chemical Review Program, and a toxicological assessment and risk management recommendations on public health aspects have previously been conducted.

In 2003 a study involving ambient air sampling on another OP, profenofos, was submitted to the APVMA for consideration. In addition to considering the impact of this information with specific regard to profenofos, the APVMA also considered that there could be implications for other chemicals, and in particular diazinon which has both high volatilisation potential and inhalation toxicity.

In May 2003 the paper *Secondhand Pesticides* was published by Californians for Pesticide Reform. In response to this paper, the APVMA requested the OCS to consider the inhalation exposure risk of diazinon.

The APVMA has now considered whether concerns are raised with respect to the current draft findings and recommendations of the diazinon review, based on results of air monitoring studies in the USA (Kegley & Katten, 2003) and the review of a study on profenofos ambient air sampling and analysis in cotton-growing areas of NSW.

Focussing on agricultural and professional pest control products and domestic household products for use inside the home, the APVMA have considered the following specific issues:

- What are the implications of the above reports on the findings and recommendations of the review of diazinon?
- Does this new information indicate that use of diazinon in enclosed areas (including homes and greenhouses) is a potential undue hazard to applicators and bystanders?
- What is the maximum quantity of active that can be applied in an enclosure before volatalisation becomes a concern?
- Can it be confirmed that volatilisation is not of concern with regard to pet collars and HG products?
- Whether any additional related information should be assessed in relation to volatilisation?

This report constitutes the preliminary assessment of the air monitoring data and product use information provided, together with estimates of human inhalational intake of diazinon based on the supplied data, additional information obtained from the Californian EPA, and the US EPA Interim Reregistration Eligibility Decision on diazinon (2002). The implications for human health are discussed, and responses provided on the issues raised by the APVMA.

1.2.2 Outdoor agricultural use of diazinon

Mongar KM, Castronovo CL & Lew G (1998): Report for the application (Kings County) and ambient (Fresno County) air monitoring of diazinon during winter 1998. California Environmental Protection Agency Air Resources Board, Engineering and Laboratory Branch, Monitoring and Laboratory Division Project Nos: C97-070 (Application) & C97-069 (Ambient) Report date: November 6, 1998 Published at http://www.cdpr.ca.gov/docs/empm/pubs/tac/diazinon.htm

Data from the application site monitoring part of this study were summarised and cited in:

Kegley S, Katten A & Moses M (2003) Secondhand Pesticides: Airborne pesticide drift in California Californians for Pesticide Reform, Report Date: May 7, 2003 Published at http://www.panna.org

Methods

Airborne diazinon levels were monitored over a 3-d period adjacent to an orchard treated with diazinon by ground spray. The application conditions are given in Table 1:

Table 1. Table 1: Application conditions

Location & date	Kings County, California USA, January 27, 1998
Treated area	Peach orchard (dormant), 16.2 ha
Time	09:30 – 13:30
Product applied	*Clear Crop Diazinon 50W* (Active = diazinon, 50%)
Application rate	2.24 kg diazinon / ha (Total applied = 36.3 kg)
Application volume	1869 L water / ha
Diazinon concentration in spraymix	1.2 g/L
Additional products / constituents in spraymix	• *Clear Crop Super 94 440 Spray Oil*, at 1:50 in spraymix • *DuPont Asana XL* (Active = esfenvalerate, concentration unknown) at 0.3 g product/L • *Micro Flo Nu-Cop 50 DF* (Active unidentified) at 6 g product/L
Application method	Described as "ground rig spray (blower)"; no other details were provided; droplet size unknown

[Comment: The above situation, application rate and diazinon concentration in the spray mixture are comparable to the use pattern of diazinon in Australia. Label directions for an Australian-registered 800 g/L diazinon product include use on orchard crops by high volume spray at 30 – 125 mL formulation/100 L water, which equates to a concentration range of 0.24 – 1.0 g active constituent/L. However, the application rate/ha in orchards is unknown. The same Australian 800 g/L product is also used on field crops at 75 mL – 1.4 L/ha and on vegetables at up to 5 L formulation/ha, depending on the situation and application method (see Appendix). Hence, the application rate in these circumstances could attain 4 kg diazinon/ha.]

Atmospheric samplers were positioned to the West, North, East and South of the treated orchard at approximate distances of 22, 23, 15 and 22 m, respectively. Atmospheric samples were collected by drawing ambient air through XAD-2 resin tubes at a flow rate of 3 L/min.

The sampling system was operated continuously throughout the study. The resin tubes were protected from direct sunlight and rain and were supported at about 1.5 m above the ground. Prior to diazinon application, background samples were taken from each position to establish whether any diazinon from other treatments was detectable. Samples were then collected during application (+ 1.5 h), followed by a 2-h sample, a 4-h sample, an 8.5-h sample, a 7.5-h sample, a 23-h sample and a 24-h sample (see Table 2).

Meteorological conditions (wind speed and direction, relative humidity and air temperature) were monitored continuously throughout the study from a station positioned at the Southwest edge of the orchard. Data were collected at a height of approximately 6 m. The skies were overcast during the background sample collection, clear throughout the first 15 h post-application, and were overcast with occasional rain and fog throughout the remaining sampling period.

Table 2. Table 2: Monitoring conditions and protocol

Sample	Time interval (h) from start of application	Predominant wind direction & speed (m/s)	Barometric pressure (hPa)	Temperature (°C)
Background	-16.5 - 0	SE, 0 – 2.9	1005 - 1007	7 - 13
Application	0 – 5.5	SE, 0 – 3.0	1004 - 1007	10 - 19
Period 2	5.5 – 7.5	N/SE, 0 – 0.04	1004	17 - 20
Period 3	7.5 – 11.5	SW, 0 – 0.5	1004 - 1005	10 - 16
Period 4	11.5 - 20	NW, 0 – 1.3	1005 - 1006	5 - 10
Period 5	20 – 27.5	SE, 0 – 2.8	1002 – 1005	4 - 15
Period 6	27.5 – 50.5	SE, 0 – 5.4	998 - 1003	12 - 17
Period 7	50.5 – 74.5	E, 0 – 7.7	1002 - 1007	3 - 16

At the end of each sampling period, resin tubes were capped, placed in culture tubes and transported on dry ice to the laboratory, where they were extracted and analysed immediately or after frozen storage. The resin tubes were spiked with 500 ng diazinon D-10 (≥99% purity, from Cambridge Isotope Laboratories) and then desorbed with 2.5 mL ethyl acetate. Diazinon concentration in the extract was measured by gas chromatography (mass selective detector). Airborne diazinon concentrations were calculated from the quantity of extracted diazinon divided by the volume of air sampled (see Table 3). For a 24-h sampling period, the minimum detection limit and estimated quantitation limit were 2.1 and 10 ng/m^3, respectively. Appropriate blanks and controls were also run. However, although the authors demonstrated a 45-74% recovery of diazinon from sample tubes under field conditions (except an outlying example from which there was only 7% recovery), it is unclear whether or how the analytical results were corrected for loss of diazinon during sampling, storage, extraction and assay.

1.2.3 Results

The background data suggest that diazinon had been applied elsewhere in the vicinity of the study orchard prior to treatment. However, the background concentrations were two orders of magnitude lower than the highest diazinon levels, which occurred between 6 and 7 h post-application (ie. during Periods 2 and 3; see Table 3). The highest recorded concentrations were therefore attributable to volatilisation of applied diazinon, rather than from spray aerosols. The maximum diazinon concentration measured was 5500 ng/m^3. Airborne diazinon levels decreased to 25 – 40% of peak values during the night after application but rose again at some locations the following day, due to increased wind speed. Wind direction

also had a significant influence on airborne diazinon concentrations, which were, as expected, highest in samplers located downwind of the test orchard. The highest levels were consistently attained at the Western sampling site. During the 3rd and final day of the study, airborne diazinon levels declined to 600 ng/m^3 to the West of the application site but were 170 ng/m^3 or less in the other directions.

Table 3: Airborne diazinon concentrations (ng/m^3) during and after application

	North sampler	East samplers*	South sampler	West sampler
Background	75	29	28	34
Application	3100	1500	870	3800
Period 2	2900	1750	1400	5200
Period 3	3500	3000	3400	5500
Period 4	800	1200	1300	1500
Period 5	150	117	170	3000
Period 6	940	87	58	2400
Period 7	170	125	140	600

*Mean result from 2 co-located samplers
See Table 2 for times the different periods covered

1.2.4 Discussion

Estimation of bystander exposure to diazinon

At issue is whether the above data raise concerns about inhalational exposure of bystanders (residents of nearby farms and rural townships) to diazinon. Based on the above monitoring, a representative atmospheric concentration range of 1500 - 5500 ng/m^3 may be set, during the first 24 h at distances up to 25 m from the spray zone.

Inhalation exposure may be estimated as follows:-

Representative atmospheric concentration range	1500 - 5500 ng/m^3	
Body weight	- adult	70 kg
	- child, age 6	20.8 kg
Inhalation rate (moderate activity/resting)	- male	2.5/0.7 m^3/h
	- female	1.6/0.3 m^3/h
	- child, age 6	2.0/0.4 m^3/h
Percentage absorption through lungs (default)	100%	

NB: The above exposure factors are US EPA recommended values for use in risk assessments.

Table 4: Estimate of bystander inhalation intake of diazinon over 24 hours during and after application

	Inhalation rate (m^3/h) (mod/rest)	24-h air intake (m^3)*	Diazinon intake (µg/d)**	Body weight (kg)	Diazinon intake (µg/kg bw)
Adult male	2.5/0.7	31.2	47 - 172	70	0.67 – 2.46
Adult female	1.6/0.3	17.6	26 - 97	70	0.39 – 1.39
Child, age 6	2/0.4	22.4	34 - 123	20.8	1.63 – 5.91

*Intake calculated on the basis of 8 h 'moderate' and 16 h 'resting' inhalation.
**No data are available on the extent to which diazinon is absorbed across the lungs, and so 100% absorption is assumed.

Comparison of intake with an intake level without harm to human health

The Australian ADI for diazinon is 0.001 mg/kg bw/d, derived from a NOEL of 0.020 mg/kg bw/d in a 37-43 d human study (Lazanas *et al.,* 1966), using a 20-fold safety factor to account for the closeness of the NOEL and LOEL (for plasma ChE inhibition) of 0.025 mg/kg bw/d.

The Australian ARfD for diazinon is 0.01 mg/kg bw, based on a NOEL of 0.2 mg/kg bw in a single-dose human study (Boyeson, 2000) in which significant RBC ChE inhibition was observed at the next highest dose of 0.21 mg/kg bw. A safety factor of 20 was applied to the NOEL, chosen because of the limited nature of the study and the closeness of the NOEL and LOEL.

Given that outdoor spray application of diazinon in an agricultural setting would probably cause an intermittent pattern of bystander exposure, it is more appropriate to compare estimated systemic intakes of diazinon with the ARfD than the ADI.

Thus, it can be seen that the estimated systemic intakes of diazinon via inhalation range between 7 and 25% of the ARfD for adult males, 4 and 14% for adult females, and 16 – 59% for children. Although none of these estimates exceeds the ARfD, there is clearly a narrow margin between the ARfD and the upper range of the exposure estimate for children. The magnitude of the estimated exposure is therefore at the limit of acceptability from a public health viewpoint, provided there was no more than occasional episodes of exposure. Furthermore, because the estimated systemic intakes exceed the ADI by 250, 140 and 590% for men, women and children, respectively, it would not be acceptable for the public to be so exposed on a regular or frequent basis.

It should be emphasised, however, that to achieve systemic intakes of diazinon equating to the ARfD and/or exceeding the ADI, bystanders would have to remain continuously for 24 h within 25 m of the application site. This situation is unlikely to arise unless residences were located immediately adjacent to a treated field. Most bystander exposure would probably occur at greater distances, but the application data from the present study can not be used to predict the airborne diazinon concentrations to which more distant bystanders would be exposed.

Ambient monitoring data from the Air Resources Board study [not assessed here] demonstrated peak diazinon levels of 40 ng/m^3 in urban air and 160 ng/m^3 at a rural school located "several hundred yards" from stonefruit orchards. If the same method is used to

calculate the amount of diazinon that would be absorbed by a 6-yr old child over a 24-h period of exposure at 160 ng/m^3, the intake would be 3584 ng/d, leading to an estimated systemic dose of 0.17 µg/kg bw/d. This dose is equivalent to 1.7% of the ARfD and 17% of the ADI. Thus, while the Californian ambient data are limited by a lack of information on the concurrent use of diazinon in the relevant districts, it supports the view that outdoor agricultural application is unlikely to expose bystanders to toxicologically significant doses of diazinon at distances of several hundred metres.

Estimation of Occupational Exposure to Diazinon

Again, based on the above monitoring, a representative atmospheric concentration range of 1500 - 5500 ng/m^3 may be set, during the first 24 h post-application at distances up to 25 m from the spray zone. However, it is assumed that occupational exposure would be limited to an 8-h working period.

Inhalation exposure may be estimated as follows:-

Representative atmospheric concentration range	1500 - 5500 ng/m^3	
Body weight	- adult	70 kg
Inhalation rate (moderate activity)	- male	2.5 m^3/h
	- female	1.6 m^3/h
Percentage absorption through lungs (default)	100%	

Table 5: Estimate of worker inhalation of diazinon over 8 hours during and after application

	Inhalation rate (m^3/h)	8-h air intake (m^3)	Diazinon intake (µg)*	Body weight (kg)	Diazinon intake (µg/kg bw)
Adult male	2.5	20	30 - 110	70	0.43 – 1.57
Adult female	1.6	12.8	19 - 70	70	0.27 – 1.00

*Assuming 100% absorption through the lungs.

Comparison of intake with an intake level without harm to human health

It can be seen that for persons working approximately 25 m from a treated outdoor site for 8 h, the estimated systemic intakes of diazinon via inhalation range between 4 and 16% of the ARfD for adult males, and 2.7 and 10% for adult females. These doses are not of toxicological concern provided the pattern of exposure is discontinuous, but would not be acceptable if exposure occurred on a daily basis for an extended period, as there is clear potential for both men and women to receive a systemic dose at about the level of, or exceeding the ADI (1 µg/kg bw/d).

It should be noted that the data from this application monitoring study cannot be used to predict the airborne concentration of diazinon or potential human exposure at closer distances to or within the application site, or when diazinon is applied by methods other than ground rig spray (blower).

Comparison of intake with studies on experimental animals

Some additional insight into the possible toxicological implications of airborne diazinon for humans may be obtained from relevant experimental data. In a 21-d inhalational toxicity study, rats exposed (nose only) to aerosols of diazinon at concentrations of 0.05, 0.46, 1.57, or 11.6 mg/m^3 for 6 h/d, 5 d/wk for 21 d showed no clinical signs of toxicity, but females had significant dose-related reductions in brain, erythrocyte and plasma ChE activities at concentrations $\geq$0.46 mg/m^3. Other findings at higher concentrations were reduced serum glucose concentration in males at 1.57 and 11.6 mg/m^3 and reduced RBC and Hb, and increased MCV in females at 11.6 mg/m^3 (Hartmann, 1990).

Thus, there were no biologically significant treatment-related effects in rats exposed repeatedly to diazinon at 0.05 mg/m^3 (50000 ng/m^3), which is approximately 9-fold higher than the peak concentration detected in the application air monitoring study.

If the systemic dose in rats at the inhalational NOEL is calculated (assuming 100% inhalation absorption, an inhalation rate in rats of 0.29 m^3/d [or 0.073 m^3/6 h] and a bw of 0.35 kg), the diazinon intake at 0.05 mg/m^3 was 0.01 mg/kg bw/d. This is the same as the ARfD for humans. At the LOEL of 0.46 mg/m^3, the estimated systemic dose would have been 0.096 mg/kg bw/d, which is similar to the LOEL of 0.12 mg/kg bw for plasma ChE inhibition in humans following a single oral dose of diazinon (Boyesson, 2000) (see Table 6).

Therefore, it appears that the highest estimated inhaled systemic dose for human bystanders (0.0059 mg/kg bw) is approximately 6% of the threshold inhaled dose for ChE inhibition in rats. Although only limited inferences may be drawn, due to the fact that the rats were subjected to repeat (rather than single) exposure, comparison between the data from humans and short-term studies in rats does not reveal any grounds for concern that either bystanders or workers are likely to experience toxicologically significant ChE inhibition resulting from occasional exposure to diazinon at the levels monitored in California.

Table 6: Comparison between NOELs and threshold doses for ChE inhibition in short-term studies with diazinon

Study/species	NOEL (mg/kg bw/d)	LOEL (mg/kg bw/d)	Reference
Human oral single dose	0.20*	0.21 (RBC ChE inhibition)	Boyeson 2000
Human oral 37-43 d	0.02	0.025 (plasma ChE inhibition)	Lazanas *et al.* (1966)
Rat oral (diet) 7 d	Not established	0.2 (plasma ChE inhibition)	Davies & Holub (1980)
Rat oral (diet) 28-d	0.02	2.3 (plasma & RBC ChE inhibition)	Chang (1994)
Rat inhalation 21-d	0.01^	0.1^ (plasma, RBC & brain ChE inhibition)	Hartmann (1990)

*Plasma ChE inhibition occurred at and above 0.12 mg/kg bw but not at 0.03 mg/kg bw.
^Estimated systemic dose.

1.2.5 Home garden and veterinary uses of diazinon

In 2000, the US EPA released an Occupational and Residential Exposure Assessment of diazinon. Based on this assessment, the Agency concluded that all residential applicator and post application scenarios posed risks of concern to applicators and children (US EPA 2000, 2002). Consequently, all residential indoor and outdoor uses (including pet collars) were phased out and no diazinon products with residential uses will be sold in the USA after December 31, 2004. The US regulatory action was based in part on estimates of inhalation exposure calculated using the results of air monitoring studies, but also included estimates of dermal and oral exposure.

1.2.6 Diazinon use within enclosed spaces

Home garden (HG) and professional diazinon products are registered in Australia for insect control within domestic premises, other residential accommodation, industrial buildings including food processing plants, and ships, aircraft and railcars (see Appendix).

A 38 g/L household ready-to-use spray is intended for indoor application to window sills, entry points, runs, forage areas, skirting boards, carpets, cracks/crevices, and under appliances. An 800 g/L product is intended for application by swingfog, spray or mister in farm, commercial, and industrial settings, and by spray or mister in homes, hotels, bakeries and canteens. Specified dilution rates are 6 mL/L for indoor sprays, 15 mL/L for indoor misting and 60 mL/L for swingfogging. However, there is no information on either product which would enable estimation of the amount of diazinon that would be applied per unit volume or surface area.

More extensive label instructions for use are provided for a professional product containing 240 g/L microencapsulated diazinon. The concentrate should be applied at 210-420 mL in 10 L water by spray or brush to drawers, shelves, storage areas, under cabinets or appliances, baseboards, window frames, wall voids, crawl spaces, trails, and cracks/crevices. A 420 mL/10 L dilution (equivalent to 10 g diazinon/L) is recommended for spray application to pet bedding, floor coverings and soft furniture at a rate of 1.25-2.5 L spraymix/100 m^2. Treatment of a 100 m^2 area would therefore result in deposition of 12.5 – 25 g diazinon at a mean surface concentration of 0.125 – 0.25 g/m^2.

The use pattern and application rate of the above microencapsulated product are similar to those described in the US EPA's analysis of air monitoring studies performed by Novartis (1980, 1981) and a further experiment undertaken by the North Carolina State University (Wright & Leidy, 1982). [Evaluated by US EPA as a combined study by Lunchick (1997); MRID No. 443488-01] According to the US EPA Health Effects Division (HED) assessment report (2000), the Novartis studies involved application of 10-11 g diazinon to whole houses as a crack and crevice treatment. The product employed was a 4 lb/gal (47.5% diazinon) emulsifiable concentrate. Wright & Leidy monitored the air within a 45 m^3 dormitory following application of 1.9 g diazinon (product unknown). In Table 16, p 85 of the HED report, the US EPA present a tabular summary showing that the average maximum and 24-h post-application airborne diazinon levels measured in the 3 studies were 54 and 22 $\mu g/m^3$, respectively.

Estimation of residents' exposure from inhalation

Utilising the mean 24-h post-application airborne diazinon level from the data evaluated by US EPA (viz. 22 μg/m^3), and the assumptions for bystander exposure detailed above, a 24-h inhalation intake for residents may be estimated as follows:

Table 7: Estimate of residents' inhalation intake of diazinon over 24 hours after application

	Inhalation rate (m^3/h) (mod/rest)	24-h air intake (m^3)*	Diazinon intake (μg/d)**	Body weight (kg)	Diazinon intake (μg/kg bw)
Adult male	2.5/0.7	31.2	686	70	9.80
Adult female	1.6/0.3	17.6	387	70	5.53
Child, age 6	2/0.4	22.4	493	20.8	23.7

*Intake calculated on the basis of 8 h 'moderate' and 16 h 'resting' inhalation at 22 μg/m^3.
**No data are available on the extent to which diazinon is absorbed across the lungs, and so 100% absorption is assumed.

Comparison of intake with an intake level without harm to human health

By comparison with the ARfD, which is the most appropriate toxicological benchmark due to the anticipated intermittent pattern of resident exposure, the estimated systemic inhalation doses for adult males and females are 98 and 55% of the ARfD for diazinon, respectively. The estimated dose for a child is 237% of the ARfD, which is unacceptable from a public health standpoint and raises concern over the indoor use of products containing diazinon.

It should be noted that these particular dose estimates may be less than the actual achieved systemic doses, as they do not take potential dermal or oral exposure into account. Additional exposure via the dermal route is highly probable, and may be significant, particularly for children and infants making contact with diazinon on treated carpets and furniture.

In the absence of the studies themselves, it is not possible to comment on study design factors which may have influenced the results. These would include the internal volume of the treated houses, the time interval between application and commencement of air sampling, laboratory methods, and the extent to which the treated premises were ventilated. It is also unclear whether higher airborne levels of diazinon would be achieved after surface application than following treatment of cracks and crevices. It is therefore essential that a more refined estimate of post-application exposure is performed based on an evaluation of the original studies by Novartis (1980 and 1981), Wright & Leidy (1982), and any other available relevant data, including human biomonitoring studies. Additional information is required on the amount of diazinon that would be released when applied within enclosed areas by misting or swingfogging, or from HG products intended for indoor use. Given that both microencapsulated and non-encapsulated diazinon formulations are used within buildings, it is desirable to confirm whether microencapsulation influences the volatilisation of diazinon. It would also be desirable to confirm whether any of the Australian-registered dust formulations are intended for indoor use, as these may cause inhalation and/or dermal exposure to humans both during and after application.

Estimation of occupational exposure from inhalation

Utilising the mean maximum airborne diazinon level from the data evaluated by US EPA (viz. 54 μg/m^3), and the assumptions for occupational exposure detailed above, an 8-h inhalation intake for spray applicators may be estimated as follows:

Table 8: Estimate of worker inhalation intake of diazinon over 8 hours

	Inhalation rate (m^3/h) (mod)	8-h air intake (m^3)*	Diazinon intake (μg/d)**	Body weight (kg)	Diazinon intake (μg/kg bw)
Adult male	2.5	20	1080	70	15.43
Adult female	1.6	12.8	691	70	9.87

*Intake calculated on the basis of 8 h 'moderate' inhalation at 54 μg/m^3.
**No data are available on the extent to which diazinon is absorbed across the lungs, and so 100% absorption is assumed.

Comparison of intake with an intake level without harm to human health

It can be seen that for women, the estimated inhalational intake of diazinon would be approximately equivalent to the ARfD of 10 μg/kg bw, while the estimated intake for men would exceed the ARfD by 50%. Hence, it would be of concern from a toxicological viewpoint if operators were exposed to airborne diazinon without respiratory protection for an 8-h workday at 54 μg/m^3, the mean peak concentration measured in the US monitoring studies. [Currently, respiratory protection is not recommended for any diazinon product covered in the Handbook of Safety Directions, First Aid Instructions and Warning Statements for Agricultural and Veterinary Chemicals.]

Again, however, it is essential to obtain the original studies cited by the US EPA. This will permit detailed examination of data and the numerous design factors that could have influenced the reported peak airborne diazinon concentrations. Any other available relevant data, including human biomonitoring studies, should also be assessed before a definitive estimate of operator exposure can be calculated. Some further aspects must also be considered, including the most probable duration and pattern of operator exposure when applying diazinon products indoors, and whether microencapsulation would influence the volatilisation of diazinon from aerosols.

At present, the APVMA has no detailed information the on the amount of diazinon that would be applied in greenhouses, or in buildings and other enclosed areas by misting or swingfogging. It is therefore not possible to estimate the potential for exposure to airborne diazinon under these circumstances. Given that the preliminary estimate suggests that operators or persons re-entering treated enclosed areas could indeed inhale diazinon at above safe doses, detailed assessment of these use patterns is required.

Diazinon in flea collars

The relevant label is for a 40 g collar containing 150 g diazinon/kg and having a 5 month effective life. If the collar contains a total of 6 g diazinon and all the active constituent is released at a constant rate over 150 d, then the release rate would be 0.04 g/d.

In the study of Wright & Leidy (see previous section), a peak airborne concentration of 38 μg/m^3 was measured when 1.9 g diazinon was applied in a 45 m^3 room. Assuming a linear relationship between the mass of diazinon and the achieved airborne concentration, a 0.04 g quantity of diazinon in a similar sized room would cause an airborne concentration of approximately 0.8 μg/m^3.

The most conservative estimate of potential inhalation intake would involve a child remaining continuously in a small room with a collared pet for 24 h. Using the methods elaborated previously, the following result is obtained:

Table 9: Estimate of a child's inhalation intake of diazinon over 24 hours from a flea collar in a 45 m^3 room

	Inhalation rate (m^3/h) (mod/rest)	24-h air intake (m^3)*	Diazinon intake (μg/d)**	Body weight (kg)	Diazinon intake (μg/kg bw)
Child, age 6	2/0.4	22.4	17.9	20.8	0.86

*Intake calculated on the basis of 8 h 'moderate' and 16 h 'resting' inhalation at 0.8 μg/m^3.
**No data are available on the extent to which diazinon is absorbed across the lungs, and so 100% absorption is assumed.

The estimated systemic dose of 0.85 μg/kg bw is 85% of the ADI and 8.5% of the ARfD for diazinon, suggesting that inhalation exposure to diazinon from flea collars is not of concern. In practice, the release estimate from the collar is very conservative, and furthermore, a child is very unlikely to remain in a confined area with a pet for 24 h. Hence, an 0.85 μg/kg bw inhalation dose is unlikely to be attained, even if higher amounts of diazinon were released from a freshly applied collar.

Diazinon use on turf

Product labels include products for insect control on domestic lawns (see Appendix). One of these is a HG EC containing 200 g/L diazinon. Its recommended dilution rate is 150 mL product/10 L water, to be applied by spray at 10 L mixture over 100 m^2 lawn. The second product is a microencapsulated flowable formulation available in HG and professional pack sizes, containing 240 g/L diazinon. Label directions specify spray application on domestic lawns at 15 – 30 mL/10 L over 10 m^2 [HG pack] or at 125 – 300 mL/15 L over 100 m^2 [professional pack]. Based on the label directions, the application rate of diazinon would be approximately 0.3 – 0.7 g/m^2.

The US EPA HED assessment of diazinon includes a summary of a residential exposure study (Rosenheck, 1999; MRID 44959101) in which a liquid diazinon product was applied by spray to turf at 4.4 lb active constituent/acre (0.5 g/m^2). Air samples were collected 1 m above ground level for 8 h post-application. The average air concentrations detected were 3.66 and 1.34 μg/m^3 over 0-2 h and 2-4 h, respectively, and 2.49 μg/m^3 over 0-4 h. Data for 4-8 h were not reproduced. Diazinon residues on turf were also measured.

The airborne diazinon concentrations during the first 4 h following application to turf are an order of magnitude lower than those detected within homes, and similar to the concentrations adjacent to the treated orchard featuring in the Californian air monitoring study. Hence, the limited available data suggest that if diazinon were to persist at the average peak concentration of 3.66 μg/m^3 for 24 h, the post-application inhalation dose for residents would approach but not exceed the ARfD.

However, the raw data were not presented and the upper range of the measured airborne diazinon concentrations is not available. It cannot be exclude that a potential for some individuals to be exposed to significantly higher airborne diazinon levels than the mean peak concentration of 3.66 μg/m^3 exists. It would be desirable to obtain the data from 4-8 h post-application, which would yield improved information on the decline of diazinon levels with time. The post-application residential exposure study of Rosenheck (1999) should be evaluated to enable a reliable conclusion as to the toxicological significance of exposure to diazinon following turf treatment. Assessment of this study should include intake of diazinon via the dermal route, as post-application exposure to residues on grass is possible.

Furthermore, two other studies should be evaluated: a biomonitoring study by Rosenheck (2000) [assessed by the US EPA as MRID 45184305] and an Occupational and Residential Exposure Task Force study [Klonne 1999; MRID 44972201]. Both these additional studies measured inhalation and dermal intake of diazinon by residents during application of lawn care products, using passive dosimetry and/or measurement of urinary metabolites.

1.2.7 Conclusions

Outdoor agricultural use of diazinon

The Californian EPA air monitoring study (Mongar et al., 1998, cited in Kegley et al., 2003) was performed under conditions similar to the Australian use pattern in orchards, pasture and field crops, and is considered relevant to public health risk assessment in this country. The study showed that significant amounts of diazinon were transported by air to a distance of approximately 25 m beyond the boundary of the treated orchard, probably by spray drift during the application period and volatilisation during the subsequent 3 days. A conservative estimate of diazinon intake, conservatively assuming continuous bystander exposure at approximately 25 m from the application site for 24 h during and after application, shows that a child could inhale up to 59% of the ARfD for diazinon. Due to their lower inhalation volume per unit bw, adult males and females would receive up to 25 and 14% of the ARfD. The estimated doses for men, women and children exceed ADI for diazinon by 250, 140 and 590%, respectively.

Given that the ARfD is the highest dose that can be ingested on a single day without appreciable risk to humans, the estimated inhalation intakes would not be expected to cause toxicity provided that there were only occasional episodes of exposure. However, due to the fact that the estimated inhalation intakes exceed the ADI for diazinon, it would not be acceptable for bystanders to be exposed at the measured airborne levels on a regular or frequent basis.

The probability that outdoor agricultural use of diazinon is likely to have a harmful effect on the public depends on the distance between bystanders and the application site. It is considered that the majority of bystander exposure would occur at much greater distances

from treated sites than the 25 m from which the monitoring was performed. The data on airborne diazinon levels several hundred metres from a Californian orchard district showed that even at the highest concentration detected, the estimated 24-h inhalation dose for a child would not exceed 17% of the ADI, which is not of concern from a toxicological standpoint.

Therefore, based on the available air monitoring data, it is considered that drift and volatilisation of diazinon from outdoor agricultural ground rig spraying of crops is unlikely to present a health hazard to the public, provided that persons residing immediately adjacent to treated areas are not exposed more often than several times per year.

With respect to occupational exposure, the estimated inhalation intake of diazinon by persons working an 8-h day at approximately 25 m from a treated outdoor site is no more than 17% of the ARfD. This is unlikely to present a health hazard to workers provided there was a discontinuous pattern of exposure.

The available data does not permit these conclusions to apply to aerial spraying or in other situations that may increase the potential for diazinon to enter the atmosphere outside the treated area.

Diazinon use within enclosed spaces, including homes

A number of registered uses for diazinon were not considered in the toxicology reviews of July 1999 or December 2002. These uses include application by spray, misting and/or swingfogging within homes and other residential accommodation, industrial premises including food production plants, greenhouses, ships, aircraft and railcars.

Data on airborne diazinon concentrations during and after indoor use of diazinon have been analysed by the US EPA (2000, 2002). Based on summary data presented in the EPA HED Occupational and Residential Exposure Assessment, it is estimated that the inhalation doses for male and female residents, respectively, may approach 98 and 55% of the ARfD for diazinon, during the 24 h following application of diazinon to cracks and crevices. The estimated dose for a child could be up to 237% of the ARfD, which is unacceptable from a public health standpoint. Furthermore, the estimated inhalation uptake for a male worker is 150% of the ARfD for diazinon, assuming 8 h exposure at the measured peak airborne concentration during application. The estimated inhalation dose for a female worker is equivalent to the ARfD.

Consequently, the indoor application of diazinon in homes may result in an undue hazard to both residents and applicators. Therefore, it is proposed that a detailed scientific assessment should be undertaken of studies on the potential for human exposure to diazinon following its use in homes and other enclosed spaces. The assessment should encompass the data reviewed by the US EPA (listed below) and any other available relevant studies, and would preferably include an estimate of dermal uptake of diazinon, given that at least one product is intended for application to carpets and soft furnishings.

To facilitate the proposed assessment, further information (including application rates) will be considered on the use of diazinon in greenhouses, and on the amount of diazinon that would be applied within houses, non-residential buildings and other enclosed structures by misting and swingfogging.

An estimate of the maximum quantity of diazinon that can be applied in an enclosure before volatilisation becomes a concern, could not be determined at this stage because there is insufficient information to support a reliable estimate. While it may be possible to predict the airborne concentration that would arise from deposition of a known quantity of diazinon within an enclosure of known volume, volatilisation would be influenced by numerous additional factors including the ambient temperature, evaporation rate of the aerosol being applied, and the strength and extent of adsorption of diazinon onto the surfaces with which the chemical came into contact. It is also possible that microencapsulated diazinon may volatilise to a different extent (and over a different time interval) than diazinon that is not contained within microcapsules. This particular issue cannot be resolved without appropriate data. Furthermore, the toxicological hazard posed by airborne diazinon depends not only on its atmospheric concentration but also on the duration over which humans are exposed, which will vary between different occupational and residential settings.

Diazinon use within mushroom housing

The OCS was requested to address the use of diazinon on mushrooms and it concluded that the above considerations are likely to be applicable for this use pattern. On this basis the use of diazinon on mushrooms as per the current label directions would be of concern from a toxicological perspective if operators were exposed to airborne diazinon without respiratory protection, it was concluded that existing label instructions are inadequate. The OCS note that the proposed restricted directions for use, (24g a.i./10L water/tonne casing material, applied as a spray over the top of the casing soil immediately after casing), are likely to reduce operator exposure although there are no data available to clearly determine this. Given these findings, the OCS considers that the risks of inhalation exposure for workers applying diazinon to mushroom casings and undertaking re-entry activities must be mitigated in order for the ongoing use on mushrooms to be supported.

In order to mitigate these risks the following label instructions are proposed.

Safety directions:

When preparing spray and using the prepared spray wear cotton overalls buttoned to the neck and wrist, a washable hat, elbow length PVC gloves and a full facepiece respirator fitted with combined dust and gas cartridge.

Re-entry:

Do Not re-enter treated areas or re-handle treated mushrooms for 14 days after treatment. If entry to treated areas is required for watering of beds, or monitoring of carbon dioxide levels, workers must avoid contact with treated casings and wear cotton overalls buttoned to the neck and wrist, a washable hat, elbow length PVC gloves and full facepiece respirator fitted with combined dust and gas cartridge. Clothing must be washed after each day's use.

Re-entry after 14 days.

The mushroom housing MUST be adequately ventilated by mechanical means (complete air replacement) prior to commencement of normal activities

Diazinon in flea collars

A conservative estimate of inhalation intake has indicated that neither the ADI nor the ARfD would be exceeded even if a child were to remain continuously for 24 h within a small room with a pet wearing a diazinon-based flea collar. Given that the normal household activity

pattern would reduce inhalation exposure to lower than estimated, the OCS can advise that volatilisation of diazinon from flea collars is unlikely to be hazardous to pet owners.

Diazinon use on turf

Several labels for products intended for application to domestic lawns by the householder or professional user were considered. According to the US EPA (2000) HED Assessment, airborne diazinon concentrations over the 4 h after turf treatment were similar to those detected adjacent to a treated orchard monitored in the Californian EPA study. The OCS therefore believes that domestic turf treatment could expose household residents to inhalation doses of diazinon approaching the ARfD, but considers that the available information is inadequate to enable a reliable conclusion. Hence, it is proposed that a detailed scientific assessment of the relevant studies evaluated by the US EPA, as listed below. The assessment should include studies on applicator exposure and on the potential for dermal exposure to diazinon residues on treated turf.

Additional information

It has been determined that the following additional related information is required for assessment of bystander, resident and occupational exposure to diazinon:

- Any available study on the absorption of diazinon via the lungs.

- Information on the use pattern and application rate of diazinon products intended for agricultural use in greenhouses or other enclosed areas (including relevant product labels).

- Information on the use pattern and application rate of diazinon products intended for use in residential buildings and non-residential buildings or enclosed areas by misting and swingfogging (including relevant product labels).

- Data on the comparative volatilisation of diazinon from microencapsulated and non-encapsulated formulations.

-Confirmation as to whether any Australian-registered diazinon dust products are intended for indoor use or application to pets, and, if so, copies of relevant product labels.

-The studies cited below, together with any additional relevant data held by registrants or available from the published literature:

Klonne D (1999) Integrated report for the evaluation of potential exposures to homeowners and professional lawn care operators mixing, loading and applying granular and liquid pesticides to residential lawns. Sponsor/Lab: Ricera Inc and Morse Laboratories Project No. OMA005, OMA001, OMA002. Unpublished; submitted to US EPA as MRID No. 44972201

Lunchick C (1997) Assessment of applicator exposure and residential postapplication exposure resulting from the indoor residential uses of diazinon Project No. 154-97: ABR-97031 Unpublished study prepared by Jellinik, Schwartz & Connolly, Inc Submitted to US EPA as MRID No. 44348801

Novartis (1980) and (1981): Residential indoor air monitoring studies; citations not known.

Rosenheck L (1999) Determination of transferable residues on turf treated with diazinon: final report Lab: Central California Research Laboratories Project No. 210-98: 980018: 302925 Unpublished; submitted to US EPA as MRID No. 44959101

Rosenheck L (2000) Determination of exposure during the mixing, loading and application of liquid diazinon to residential turf through the use of passive dosimetry and biological monitoring Lab: Novartis Crop Protection, Inc Project No. 767-98: I024480NAU950T Unpublished; submitted to US EPA as MRID No. 45184305

Wright & Leidy (1982): Citation not known. US EPA may be referring to Leidy et al. (1982): Concentration and movement of diazinon in air J Environmental Sci Health 17; 311-319

1.2.8 References

Boyeson MG (2000) A randomised, double-blind, ascending, acute, oral dose study of diazinon to determine the No Effect Level (NOEL) for plasma and RBC cholinesterase activity in normal, healthy subjects. Covance Clinical Research Unit Inc., 309 West Washington Av, Suite 4 East, Madison, Wisconsin 53703, USA. Unpublished.

Chang JCF (1994) Cholinesterase inhibition in 28 day feeding study in rats. Report no. F-00186. Lab: Ciba-Geigy Corp., Crop Protection Division, Environmental Health Center, Farmington CT, USA. Unpublished. *In* ECRP Review of the Mammalian Toxicology and Metabolism/Toxicokinetics of Diazinon. Therapeutic Goods Administration, Canberra Australia, December 1998.

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Hartmann HR (1990) 21-Day repeated exposure inhalation toxicity in the rat. Report No. 891205. Lab: Experimental Toxicology, Ciba-Geigy Ltd, Stein, Switzerland. Unpublished. *In* ECRP Review of the Mammalian Toxicology and Metabolism/Toxicokinetics of Diazinon. Therapeutic Goods Administration, Canberra Australia, December 1998.

Kegley S, Katten A & Moses M (2003): Secondhand Pesticides Airborne pesticide drift in California Californians for Pesticide Reform Report Date: May 7, 2003 Published at http://www.panna.org

Klonne D (1999) Integrated report for the evaluation of potential exposures to homeowners and professional lawn care operators mixing, loading and applying granular and liquid pesticides to residential lawns. Sponsor/Lab: Ricera Inc and Morse Laboratories Project No. OMA005, OMA001, OMA002. Unpublished MRID No. 44972201

Lazanas JC, Fancher OE & Calandra JC (1966) Report to Geigy Chemicals Corporation. Subacute oral toxicity study on diazinon 50W - Humans. Report No. IBT D4321. Lab: Industrial Bio-Test Laboratories, Inc. Northbrook, Illinois, USA. Sponsor: Ciba-Geigy Corp., Ardsley, New York, USA. Unpublished. *In* ECRP Review of the Mammalian Toxicology and Metabolism/Toxicokinetics of Diazinon. Therapeutic Goods Administration, Canberra Australia, December 1998.

Lunchick C (1997) Assessment of applicator exposure and residential postapplication exposure resulting from the indoor residential uses of diazinon Project No. 154-97: ABR-97031 Unpublished study prepared by Jellinik, Schwartz & Connolly, Inc MRID No. 44348801

Mongar KM, Castronovo CL & Lew G (1998): Report for the application (Kings County) and ambient (Fresno County) air monitoring of diazinon during winter 1998 California Environmental Protection Agency Air Resources Board, Engineering and Laboratory Branch, Monitoring and Laboratory Division Project Nos: C97-070 (Application) & C97-069 (Ambient) Report date: November 6, 1998 Published at http://www.cdpr.ca.gov/docs/empm/pubs/tac/diazinon.htm

Rosenheck L (1999) Determination of transferable residues on turf treated with diazinon: final report Lab: Central California Research Laboratories Project No. 210-98: 980018: 302925 Unpublished MRID No. 44959101

Rosenheck L (2000) Determination of exposure during the mixing, loading and application of liquid diazinon to residential turf through the use of passive dosimetry and biological monitoring Lab: Novartis Crop Protection, Inc Project No. 767-98: I024480NAU950T Unpublished MRID No. 45184305

US EPA (2000) Occupational and residential exposure assessment and recommendations for the reregistration eligibility decision (RED) document for diazinon United States Environmental Protection Agency Health Effects Division, Washington DC USA D270837 Dated November 30, 2000.

US EPA (2002) Interim reregistration eligibility decision for diazinon United States Environmental Protection Agency Special Review and Reregistration Division, Washington DC USA Case No. (0238) Dated July 31, 2002.

1.2.9 Appendix 1: Use patterns of diazinon products identified from approved labels

APPLICATION	PRODUCT DESCRIPTION	APPLICATION INSTRUCTIONS	COMMENTS
Flea collar for large dogs	40 g collar containing 150 g/kg diazinon	Fix round neck. Effective for 5 months.	Label warns against allowing children to play with collar. Wash hands with soap and water after handling collar.
Household insecticide surface spray	HG Spray bottle, 600 mL-1 L, containing 38 g/L diazinon	Indoors: spray window sills, entry points, runs, forage areas, skirting boards, carpets, cracks/crevices, under appliances Outdoors: spray infested areas, bins, rubbish every 3 mo	Label warns users to ventilate treated rooms or buildings before re-occupying (no time specified).
Lawn insecticide	HG EC containing 200 g/L diazinon in liquid hydrocarbons	Recommended dilution is 150 mL/10 L. Sprayed at 10 L mixture over 100 m^2 lawn.	
Lawn insecticide	Microencapsulated flowable containing 240 g/L diazinon; 250 mL and 5L pack sizes	Sprayed on domestic lawns at 15 – 30 mL/10 L over 10 m^2 [HG pack] Sprayed on domestic lawns at 125 – 300 mL/15 L over 100 m^2 [Professional pack]	
Insect control in and around residential & industrial buildings, food processing plants, aircraft & vehicles	Microencapsulated flowable containing 240 g/L diazinon	Apply at 210-420 mL in 10 L water by spray or brush to drawers, shelves, storage areas, behind & under cabinets, appliances etc. Apply at above dilution by spray to baseboards, window frames, wall voids, crawl spaces, trails, cracks & crevices Apply at 420 mL/10 L to pet bedding, floor coverings, soft furniture using 1.25-2.5 L/100 m^2 Building perimeter treatment performed at 420 mL/10L sprayed over 40 m^2	Label warns users to avoid spraying onto food, food utensils or food processing areas. Use not more than once/60 d.
Insecticide for use in pasture, field, vegetable, plantation & orchard crops, plus homes, commercial & industrial buildings, skins & hides	Liquid concentrate containing 800 g/L diazinon	Apply by swingfog, spray or mister in farm, commercial & industrial settings, & by spray or mister in homes, hotels, bakeries & canteens. Used at 6 mL/L water for indoor sprays, up to 125 mL/100L for outdoor sprays, 5 mL/L for spraying hides, 15 mL/L for indoor misting and 60 mL/L for swingfogging.	Label warns users to avoid spraydrift onto food, food utensils or food processing machinery, and to ventilate treated rooms or buildings before re-occupying (no time specified).

		Field crops: Apply by air, boom spray or mister at 75 mL – 1.4 L product/ha Plantation / orchard crops: Apply by high volume spray at 30 – 125 mL/100 L water except pineapples which require up to 3 L product/ha Vegetables: Apply by boom spray at 150 mL – 5 L product/ha, high volume spray at 30 – 140 mL/100 L water or knapsack spray at 5-30 mL/15 L water	

1 OCCUPATIONAL HEALTH & SAFETY

2.1 Executive summary

Diazinon is used to control blowfly and lice infestation in sheep. Diazinon products are applied by various application methods including, portable and fixed plunge dipping, shower dipping and hand and auto race jetting. Workers may be occupationally exposed to diazinon during mixing, loading, and applying the pesticide.

An exposure study was conducted to investigate the extent of worker exposure to diazinon and to evaluate the effectiveness of personal protective equipment (PPE) when mixing/loading, treating sheep by the five treatment methods and cleaning up after use of the product. Passive dosimetry was used to estimate total dermal and inhalation exposure.

Exposure data obtained in this study were used to determine the occupational risk to workers during mixing/loading and application. The risk is determined by a margin of exposure (MOE), which is a measure of how close the likely occupational exposure comes to the NOEL observed in an appropriate animal or human study. The risk assessment used an internal (NOEL) dose of 0.02 mg/kg bw/day from a 37-43 day human dietary study. A MOE of 20 or more was considered to be acceptable.

An acceptable MOE was identified for mixer/loaders and cleaners wearing the PPE that was used for the exposure study including a waterproof apron. However, for all modes of application – portable and fixed plunge dipping, shower dipping and hand and auto race jetting – the MOE was found to be unacceptable for workers wearing the existing label-specified PPE. Continuation of sheep treatment by all current methods therefore cannot be supported.

Options for reducing occupational exposure during application are discussed.

2.2 Background

Diazinon is a contact organophosphorous insecticide with a broad range of insecticidal activity. In products it is commonly used as an ectoparasiticide in farmed animals and as an insecticide in broadacre agricultural situations. Diazinon products are also registered for use by pest control operators and home garden/home veterinary uses. Diazinon is one of the chemicals being reviewed as part of the Australian Pesticides and Veterinary Medicines Authority's (APVMA's) Chemical Review Program.

In response to a request by the Australian Pesticides and Veterinary Medicines Authority (APVMA) for chemical specific exposure data, the National Farmer's Federation Limited has recently completed and submitted a new study entitled, "Worker Exposure to Diazinon in Australian Sheep Industries", carried out by the Centre for Pesticide Application and Safety (CPAS), the School of Agronomy & Horticulture, and the School of Animal Studies at the University of Queensland (UQ), in conjunction with NSW Department of Agriculture. The Australian Centre for

Agricultural Health and Safety (ACAHS) provided technical direction, oversight and quality control.

This report provides a summary of the study and a determination of occupational risk for those using diazinon products on sheep.

2.3 Exposure study

Worker Exposure to Diazinon in Australian Sheep Industries, Report no. v.3.1; June 2004 (Study contact = Nicholas Wood)
Prepared by:
The Centre for Pesticide Application and Safety
The University of Queensland, Gatton Campus, Gatton, QLD
In conjunction with
NSW Department of Agriculture

2.3.1 Aim of the study

The purpose of the study was to investigate the extent of occupational exposure during the external application of diazinon to sheep. The study considered exposure during mixing and loading, application and subsequent clean-up operations following five different modes of application:

- Portable plunge dipping
- Hand jetting
- Auto race jetting
- Fixed plunge dipping
- Shower dipping

The field trial protocol was also designed to evaluate the effectiveness of current label PPE in reducing pesticide exposure, although in some cases additional PPE was employed, and to assess the risk of inhalation exposure during these application processes.

The study was conducted using a protocol approved by the APVMA in consultation with NOHSC (The agvet OHS assessment group of NOHSC was integrated into the OCS in April 2004). Sheep were dipped according to the usual industry practice in the Australian sheep industry. All the volunteers involved in this study followed the conditions/restrictions listed on the product label.

Human ethics clearance for the study was obtained from the New England Area Health Authority Research Ethics Committee and animal ethics clearance for the study was obtained from the University of Queensland Research Ethics Committee and the NSW Animal Ethics Committee. All personnel involved in the study were informed of the scope of the study and their role in it. Volunteers took part in a training session, which included an OHS induction conducted by a University of Queensland OHS officer. Prior to taking part in the study all personnel signed a consent form.

2.3.2 Study design

Six sites in NSW were chosen for the study. The study volunteers were experienced in mixing/loading and applying pesticides to sheep by all of the five application techniques listed above. Thirty volunteers were directly involved in the fieldwork component of the study but only seventeen were used to obtain exposure measurements. Each of the five application methods was repeated three times in separate sessions (total 15 sessions). In each session, one volunteer prepared the working solution (mixing/loading), another five treated sheep and a seventh cleaned the treatment area and equipment. Thus, for each application method, there were three replicates for mixing/loading, fifteen replicates for application and three for cleaning up. A summary of the study design is shown in table 1.

Table 1: Study design showing the number of sessions and volunteers for each application method

Session ID	Date	Site ID	Application Method	No. of Volunteers		
				M/L	APPL.	CLEAN.
1	04-11-03	TARC	AJR	1	5	1
2	04-11-03	TARC	PPD	1	5	1
3	04-11-03	SC	PPD	1	5	1
4	05-11-03	TF	HJ	1	5	1
5	06-11-03	SC	SD	1	5	1
6	24-11-03	SC	SD	1	5	1
7	25-11-03	TARC	PPD	1	5	1
8	25-11-03	SC	AJR	1	5	1
9	26-11-03	SPC	HJ	1	5	1
10	26-11-03	SM	HJ	1	5	1
11	27-11-03	SM	FPD	1	5	1
12	01-12-03	SAR	FPD	1	5	1
13	02-12-03	TARC	SD	1	5	1
14	02-12-03	SC	AJR	1	5	1
15	04-12-03	SPC	FPD	1	5	1

M/L – Mixing/loading; APPL. – Application; CLEAN – Cleaning down; TARC=Trangie Agricultural Research Centre; SC=Stanthorpe 'Cooinda'; TF= Trangie 'Fairview'; SPC=Stanthorpe 'Pike's Creek'; SAR=Stanthorpe 'Allum Rock'; SM=Stanthorpe 'Mulgowan'; AJR=Automatic jetting race; PPD=Portable plunge dip; HJ=Hand jetting; SD=Shower dip; FPD=Fixed plunge dip.

All participants in the study wore waterproof clothing underneath the PPE listed below:

Washable cotton hat
(Full face-shield / or goggles – cited in study report but not worn in all cases)
Half face respirator
Elbow-length PVC gloves, cuff folded outwards
Cotton overalls done up to neck/wrists
Water resistant footwear/boots

Those involved in mixing/loading operations wore a waterproof full-length bib apron in addition to the label specified PPE. Dermal exposure was measured using chromatographic paper patches attached to clothing. Cotton gloves worn on hands or

inside the gloves were used to determine the amount of diazinon coming in contact with the hands. Chromatographic paper patches were also worn in respiratory masks to determine the amount of diazinon potentially inhaled.

To measure the extent of protection afforded by the PPE, patches were placed under overalls (for workers in the internal patches group). In contrast patches were placed exterior to the waterproof clothing for workers in the external patches group. Results of the external patches group enabled determination of total body exposure, thus the patches were placed exterior to all PPE.

Diazinon emulsifiable concentrate formulation of 200 g ai/L (Virbac Jetdip Sheep Jetting Fluid and Blowfly Dressing) was used in the study. The product application rates were in accordance with the label specifications, varying from 0.1 g diazinon/L (plunge and shower dips, lice control) and 0.2 g diazinon/L (plunge and shower dips, sheep blowfly control) to 0.4 g diazinon/L (jetting, sheep blowfly control).

2.3.3 Materials

Patches:

The patches were made from Whatman chromatography paper No.17, cut into 10 cm x 10 cm squares, and stapled to a protective backing of aluminium foil. Patches were affixed (either underneath the overalls or outside the overalls), on forearms, thighs, below the knees, front neck/chest, back neck and shoulders, as follows:

2 patches on the top of the external shoulders
1 patch on the back of the neck, below the lower edge of collar
1 patch on the upper chest, near jugular notch
2 patches, one on each of forearms (with/without calico)
2 patches, one on either side on the front of thighs
2 patches, one on either side just below knees (with and without calico)

For those with external patches, 2 extra patches were placed inside the socks on the lower part of the ankles, one on either side to measure feet contamination. No patches were placed on the head in either the external or internal patch groups. Head and face exposure was approximated from the shoulder, neck and back patch data of the external patch group (see Table 2). These data were also used for the internal patch group.

Some attached patches were reinforced with one layer of unbleached calico cloth to prevent them from being torn during hand jetting activity. Cotton gloves were used to measure deposition on the hands or on gloves: cotton gloves were worn outside nitrile gloves or inside the elbow-length PVC gloves. The PVC gloves were attached to the overalls with masking tape to minimise fluid leakage into the glove.

Inhalation patches:

Respiratory filters were made using 32 layers of 7 cm diameter surgical gauze discs and 7 cm diameter Whatman chromatography paper No. 4 filter paper disc. The layers of gauze and filter were held together by two 10 cm surgical gauze discs stapled around the perimeter in four places. These filters were placed in the respirators with the chromatography paper facing outward.

Field blanks:

For field blanks, a member of the team not participating in the patching or animal handling operations was 'patched' with three field blanks, one on each of the internal shoulders and one at the back below the neck. This member (sometimes a stationary frame) was asked to remain outside the working area in a place free from any contamination of diazinon. These field blanks were subjected to the same weather conditions and the same time duration of the session but without contamination.

Patch treatment:

Body patches collected from the volunteers were carefully rolled and placed into the labelled test tube, one paper per test tube. Cotton gloves were placed as a pair into labelled jars. The respiratory paper discs were placed into labelled bottles. The sealed test tubes, jars and bottles were stored in cool boxes containing multiple freezer bricks and transferred to a freezer. Samples were then transported to analytical laboratory in 'eskies' and placed in a freezer awaiting analysis (one to eight weeks).

2.3.4 Data Analysis

Acetone (25 mL for test tubes, 50 or 150 mL for jars) was added to samples, sonicated for 10 minutes and allowed to stand. Aliquots of liquid were transferred to vials for gas chromatography with flame photometric detection (GC FPD; Hewlett Packard HP6890). Samples from high-exposure areas were diluted to bring diazinon levels down to within the linear range of the flame photometric detector. The limit of detection (LOD) of the GC FPD analytical method was 1 μg/container or 10 ng/cm^2 if there is one patch (100 cm^2) per container. Where the laboratory reported value was less than the limit of detection, a value of 0.5 μg (or 0.5 ng/cm^2) was used as a default value. Total body deposition of diazinon was estimated as the sum of diazinon residues deposited on head, face, neck (front and back), chest, back, upper arms, fore arms, hands, thighs, lower legs and feet of the volunteers. Deposition on each of these body regions was determined by multiplying the amount of diazinon deposited per square cm of patch by the surface area of that body part (cm^2). The surface areas of the body regions used in this study are as follows:

Table 2: Regional Body Surface Areas (adult male)

Body Part	Deposition in ng
Head and face	(Mean of shoulder, chest and back patches) x 1300
Back of neck	(Back patch) x 110
Front of neck	(Chest patch) x 150
Chest/stomach	(Chest patch) x 3550
Back	(Back patch) x 3550
Upper arms	(Mean shoulder and forearm) x 2910
Forearms	(Arm patch) x 1210
Hands	(Glove result) x 2*
Thigh	(Thigh patch) x 3820
Lower leg	(Low leg patch) x 2380
Feet	(Foot patch) x 1310
Total	**Total of above = Total deposition (ng)**

*When only one glove is analysed. If gloves from both hands are analysed as one sample, the exposure value is not multiplied by the factor 2.

The level of protection provided by the headgear (hat) and waterproof boots is not known. As a conservative approach, zero protection was assumed from headgear and boots. Consequently, to obtain total body exposure for the internal patch group, exposure values obtained for head (and face) and feet in the external patch group were added to the body exposure (torso, limbs and hands) of volunteers in the internal patch group. And since it was not possible to match volunteers in the two groups, the arithmetic means of the exposure values obtained for head and feet for the external patch group were added to the total body deposition of each subject from the internal patch group.

2.4 Results

2.4.1 Mixing/Loading

Occupational exposure was measured during mixing/loading diazinon product for use in all five application methods. Three replicates for each of the five application methods were monitored.

For this study, volunteers mixed the diazinon poured from 20 L steel drums, into measuring jugs and then into large volume tanks (fixed or portable plunge dips) or directly into spray tanks of 1000 L plus capacity. Recharging, where required, was conducted by animal handlers or by applicators. The quantity of diazinon active ingredient (ai) mixed and applied ranged from 0.255 kg to 1.2 kg per session. The total volume of spray mixture handled per session ranged from 500 L to 7200 L depending on the treatment method.

Typical tasks carried out by mixer/loaders were:

Transport of pesticide drums
Pouring and mixing chemicals
Loading of chemicals into plunge dip tank or spray tank
Removal of empty containers from the working areas
Cleaning up of significant spills

Recording details of chemical prepared and loaded

Table 3 presents the occupational exposure to diazinon during mixing and loading diazinon product for various application methods in the absence and presence of PPE (external and internal patches).

Table 3: Diazinon deposition calculated for different body regions in volunteers performing mixing/loading

VOLUNTEERS	EXTERNAL PATCHES								INTERNAL PATCHES					
	Head (µg)	Feet (µg)	Hands (µg)	Total body excluding head, hands and feet (µg)	Total body (µg)	Diazinon solution prepared (L)	Diazinon solution used per 250 sheep (L)	Exposure (µg/amount of diazinon mixed/loaded to treat 250 sheep)	Hands (µg)	Total body excluding head, hands and feet (µg)	Total Body (µg)**	Diazinon solution prepared (l)	Solution used per 250 sheep	Exposure (µg/amount of diazinon mixed/loaded to treat 250 sheep)
1	263.2	3.28*	196842	2897	200005	2100	700	66668	0.49*	62.6	126.8	2100	700	42.3
2	7.84	3.28	122729	307	123047	7200	1500	25635	0.49	62.6	126.8	7000	1200	21.7
3	5.42	3.28	70774	864	71648	6000	1500	17912	9.76	62.6	136.0	6500	1800	37.7
4	5.42	3.28	75085	100	75193	1200	722	45241	9.51	62.6	135.8	850	755	120.6
5	5.42	3.28	96787	402	97199	2700	1304	46944	8.61	62.6	134.9	2250	1167	70.0
6	48.8	10.1	436574	144628	581262	2550	1350	307727	5.42	375.5	444.6	1900	1530	358
7	5.42	9.04	297690	84.0	297789	5000	2347	139782	74.7	62.6	201.0	6000	2254	75.5
8	5.42	3.28	109503	132	109644	1200	909	83055	1.97	62.6	128.3	1000	767	98.4
9	284.0	9.20	92049	6579	98920	1000	695	68749	11.7	123.5	199.0	1350	833	122.8
10	44.5	3.28	29423	10768	40240	1000	755	30381	1.80	80.2	145.7	1350	833	90.0
11	5.42	3.28	112208	218	112435	6500	1316	22764	3.77	62.6	130.0	1500	1079	93.5
12	171.3	3.28	992847	4441	997462	6500	1250	191820	7.95	62.6	134.2	1200	1210	135.3
13	24.5	3.28	109010	1590	110628	3350	1292	42666	2.54	87.8	154.0	3000	1000	51.3
14	5.42	3.28	99567	523	100099	1000	500	50050	1.07	66.2	131.0	750	275	48.0
15	5.42	3.28	96525	82306	178840	7000	1716	43841	3.36	91.5	158.5	5000	1716	54.4
Mean	**59.2**	**4.51**	**195841**	**17056**	**212961**			**78882**	**9.54**	**92.54**	**165.8**			**94.6**

Head and feet exposures were not measured for 'Internal patches' group. Arithmetic means of the head and feet depositions obtained in the 'External Patches' group were added to all 'Internal Patches' group volunteers to calculate total body deposition in that group (see Section 3.3).

* Where the laboratory reported value was less than the limit of detection (1 µg/container or 10 ng/cm^2), a value of 5 ng/cm^2 was used as a default value. This value was multiplied by the respective body part area to obtain deposition on that area.

** (Total body excluding head, hands and feet) + hands + deposition on head and feet obtained in unprotected workers

Comments:

For mixing and loading detectable or quantifiable levels of diazinon were found on hands of all volunteers not wearing PPE (external patches) and on 13 of the 15 volunteers wearing PPE (internal patches) (Table 3). Five volunteers also had diazinon contamination on internal patches on forearms, shoulder and thigh. As expected, the maximum contamination in the unprotected volunteers was on the hands, followed by lower legs, forearms and front neck/chest (individual body part exposure not shown).

Inhalation pads of only one of the 15 volunteers (in the external patch group) were contaminated (data not shown). All other patches recorded diazinon residue below the limit of detection. According to the notes from the 'field book', the inhalation pad showing contamination had hit ground in de-patching area.

The highest and lowest total dermal exposure levels detected during the study for mixer/loaders was 1720 mg/kg diazinon handled/day, and 71.6 mg/kg diazinon handled, respectively for those not wearing PPE and 0.82 mg/kg diazinon handled, and 0.11 mg/kg diazinon handled, respectively for those with PPE (Table 3). Inhalation exposure was not detected in mixer/loaders.

The results show that, for mixer/loaders, gloves and overalls were very effective in providing protection to hands and the body regions they covered (Table 3).

2.4.2 Application by Portable Plunge Dip

Exposure to diazinon was measured during application of the product by portable plunge dip. In this application method, sheep enter a portable plunge dip via a 'VE sheep handling machine' set at a 45^{o} angle. A typical metal portable plunge dip is 'S' or 'U' shaped, approximately 11 to 12 m long, 0.75 m wide and 1.6 to 1.8 m deep. Sheep generally become submerged on entry and swim through a section approximately 8 m long and 1.25 to 1.35 m deep. They are submerged once more in the swim section, by an individual operator, using a dunking stick. The sheep then begin a 3 to 4 m 45^{o} ascent out of the dip and then down a ramp into a holding yard. Dipping was carried out in three sessions.

Typical tasks carried out by applicators were:

Setting up and bringing down portable plunge unit
Moving the sheep that block the race entry or exit
Connecting and disconnecting hoses

The product label recommends a concentration of 0.01% diazinon in the dip solution for body lice control and 0.02% diazinon in the dip solution for blowfly control. Accordingly, one session of plunge dip treatment used 0.01% diazinon solution and two sessions used 0.02% diazinon solution. It is not stated whether recharging the dip solution was required in any of the sessions although this is unlikely given the number of animals treated in each session. The number of sheep treated per session and diazinon deposited on different body parts are shown in table 4.

Table 4: Diazinon deposition calculated for different body regions in volunteers treating sheep in portable plunge dip

VOLUNTEERS	EXTERNAL PATCHES							INTERNAL PATCHES				
	Head (µg)	Feet (µg)	Hands (µg)	Total body excluding head, hands and feet (µg)	Total body (µg)	No. of sheep treated	Exposure per 50 sheep treated (µg)	Hands (µg)	Total body excluding head, hands and feet (µg)	Total body (µg)**	No. of sheep treated	Exposure per 50 sheep treated (µg)
1	77.65	3.28*	2697.1	4742.9	7520.9	49	7674.4	5.49	62.6	240.3	50	240.3
2	9.19	3.28	1203.8	8854.9	10071.0	50	10071.0	1.23	62.6	236.0	50	236.0
3	11.61	3.28	139.1	2266.0	2420.2	50	2420.2	0.49	62.6	235.3	50	235.3
4	10.36	3.28	210.8	1746.1	1970.6	50	1970.6	0.49	62.6	235.3	50	235.3
5	12.74	3.28	1042.8	2961.7	4020.5	50	4020.5	11.2	225.9	409.3	50	409.3
6	281.2	3.28	5030.9	19652.2	24967.8	50	24967.8	0.49	62.6	235.3	50	235.3
7	284.9	3.28	3917.5	3155.4	7361.2	50	7361.2	1.23	102.7	276.1	51	270.7
8	70.24	3.28	858.13	1376.5	2308.2	50	2308.2	370.7	62.6	605.5	49	617.9
9	38.61	3.28	4882.0	1174.9	6098.8	50	6098.8	2.21	1630.9	1802.4	50	1802.4
10	7.41	3.28	1063.5	869.5	1943.7	50	1943.7	1.39	100.2	273.8	50	273.8
11	170.6	3.28	1198.5	2896.1	4268.4	49	4355.5	9.10	62.6	243.9	49	248.9
12	271.1	12.44	1676.2	3980.3	5940.0	49	6061.2	1.39	375.5	549.1	49	560.3
13	455.7	6.81	1638.5	4537.8	6638.8	49	6774.3	4.9	62.6	239.7	51	235.0
14	503.5	3.28	1237.3	9285.1	11029.2	49	11254.3	13.0	62.6	247.8	47	263.6
15	315.5	3.28	739.2	3622.9	4680.8	49	4776.3	4.10	62.6	238.9	48	248.8
Mean	**168.0**	**4.19**	**1835.7**	**4741.5**	**6749.0**		**6804.0**	**28.5**	**201.6**	**402.3**		**407. 5**

Head and feet exposures were not measured for 'Internal patches' group. Arithmetic means of the head and feet depositions obtained in the 'External Patches' group were added to all 'Internal Patches' group volunteers to calculate total body deposition in that group (see Section 3.3).

* Where the laboratory reported value was less than the limit of detection (1 µg/container or 10 ng/cm^2), a value of 5 ng/cm^2 was used as a default value. This value was multiplied by the respective body part area to obtain deposition on that area.

** (Total body excluding head, hands and feet) + hands + deposition on head and feet obtained in unprotected workers

Volunteers 1 to 5 used 0.01% diazinon in the final dip solution and volunteers 6 to 15 used 0.02% diazinon.

Comments

In this treatment method, thighs and forearms were most contaminated in all those wearing external patches (no PPE), followed by shoulders and neck/chest (individual body-part data not shown). In volunteers with internal patches, 12 of the 15 volunteers had detectable or quantifiable levels of diazinon on hands and only 4 had contamination on other regions of the body, such as forearm, shoulder and lower leg. Inhalation pads of 5 of the 15 volunteers were contaminated with very low amounts of diazinon, the highest being 6 μg.

The highest and lowest total dermal exposure levels for treating 50 sheep by plunge dip were 24.9 mg and 1.94 mg diazinon, respectively for unprotected workers and 1.77 mg diazinon and 0.23 mg diazinon, respectively for the protected workers. Gloves and overalls provided over 95% protection to workers (Table 4).

Volunteers using 0.02% diazinon solution would be expected to receive twice the amount of diazinon over their body than those using 0.01% diazinon solution. However, no difference in exposure was noted in volunteers using 0.01% diazinon solution (volunteers no. 1-5, Table 4) and 0.02% diazinon solution (volunteers no. 6-15). No explanation was provided for the similar exposure values with both concentrations of diazinon. It is possible that the large variation in exposure values between volunteers within the group precluded any differences expected between 0.01% and 0.02% diazinon users.

2.4.3 Application by Hand Jetting

Hand jetting equipment consists of a 5-8 horsepower motor and pump that delivers the jetting solution from a 1000-1500 L portable tank through a 5 m long hose to the jetting comb or headpiece. Sheep are first placed in a handling race that accommodates approximately 50 sheep. The operator then combs the jetting headpiece through the wool of the sheep starting from the back of the head down the backline, over the right rump and down the right leg, then back up and over the left rump, down the left leg, back and up and over the tail. In male sheep, a forward and backward pass with the jetting comb is also made over the prepuce area on the underside of the sheep. The pressure at the 2 mm solid stream (straight bore) nozzles of the jetting comb is 400 to 500 kPa. Two to four litres of jetting solution are applied per sheep. After application, the sheep are let out into a holding yard.

The final concentration of diazinon in the jetting solution was 0.4 g/L. Typical tasks carried out by applicators were:

Moving the sheep that block the race entry or exit
Applying jetting solution
Maintaining pumps for jetting solution

The number of sheep treated per session and diazinon deposited on different body regions are shown in table 5.

Table 5: Diazinon deposition calculated for different body regions in volunteers treating sheep by hand jetting

VOLUNTEERS	EXTERNAL PATCHES							INTERNAL PATCHES				
	Head (µg)	Feet (µg)	Hands (µg)	Total body excluding head, hands and feet (µg)	Total body (µg)	No. of sheep treated	Exposure per 50 sheep treated (µg)	Hands (µg)	Total body excluding head, hands and feet (µg)	Total body (µg)**	No. of sheep treated	Exposure per 50 sheep treated (µg)
1	5.42	23.06	11675	129230	140935	33	213537	1.15	134051	134279	36	186499
2	63.0	42.05	9659	213865	223629	35	319470	4.42	177587	177818	36	246969
3	116.3	3.28*	14793	181412	196326	35	280464	5.33	191937	192169	36	266901
4	748.6	3.28	13006	214858	228616	35	326594	3.11	162803	163032	37	220313
5	33.8	3.28	11645	179808	191490	35	273557	11.2	170724	170962	37	231030
6	16.6	149.3	8034	112830	121031	54	112065	1.89	27010	27239	54	25221
7	64.1	171.2	8137	117424	125798	54	116479	28.1	90430	90685	54	83968
8	9.71	168.6	6115	102038	108331	54	100307	1.97	48429	48658	54	45054
9	40.0	234.4	6974	114058	121307	54	112320	572.2	64410	65209	54	60379
10	319.9	3.28	11527	115479	127330	54	117898	8.20	71894	72129	54	66786
11	64.1	3.28	5508	8476	14053	53	13257	2.38	526	755	53	712
12	589.0	9.17	6657	15448	22704	53	21418	45.8	857	1129	53	1065
13	170.5	3.28	5500	14555	20329	53	19177	6.40	598	831	53	784
14	22.53	21.5	4290	5591	9927	53	9365	2.62	3280	3509	48	3655
15	231.9	8.80	8280	11517	20037	53	18903	1.89	136	365	48	380
Mean	**166.4**	**60.3**	**8787**	**102439**	**111456**		**136987**	**46.4**	**76311**	**76585**		**95981**

Head and feet exposures were not measured for 'Internal patches' group. Arithmetic means of the head and feet depositions obtained in the 'External Patches' group were added to all 'Internal Patches' group volunteers to calculate total body deposition in that group (see Section 3.3).

* Where the laboratory reported value was less than the limit of detection (1 µg/container or 10 ng/cm^2), a value of 5 ng/cm^2 was used as a default value. This value was multiplied by the respective body part area to obtain deposition on that area.

** (Total body excluding head, hands and feet) + hands + deposition on head and feet obtained in unprotected workers

Comments

The most contaminated regions in volunteers without PPE (external patches) when carrying out hand jetting were hands, thighs and lower legs. The forearms and front neck/chest also had substantial diazinon residues. The least affected areas were back, neck and shoulders. In volunteers wearing PPE (internal patches), thighs and lower legs collected appreciable amounts of diazinon, while the neck region (front and back) was fully protected (individual body part exposure data not shown). In addition, while gloves provided almost complete protection to hands, overalls were least effective, providing only 25% protection. Inhalation patch contamination that could have been from spray splashes was seen only in 5 of the 15 volunteers and was negligible (highest value 9 μg per 50 sheep treated).

The highest and lowest total dermal exposure levels detected during the study for hand jetting operators were 326 mg and 9.3 mg diazinon (per 50 sheep handled), respectively for unprotected workers and 138 mg diazinon and 0.35 mg diazinon (per 50 sheep treated), respectively for those wearing PPE (Table 5).

2.4.4 Application by Auto Race Jetting

The equipment for auto race jetting consists of a 5-8 horsepower motor and pump that delivers the jetting solution from a 1000-1500 L portable tank through a 12.5 m long hose to the auto race jetter. The auto race jetter consists of steel frame that supports 4 horizontal in-line 2 mm solid stream nozzles both above and below the sheep. The nozzles are set at approximately a 15^{o} angle towards the front of the jetting machine.

Sheep are placed in a handling race that accommodates approximately 50 sheep and one by one they are allowed to enter the auto jetter. The sheep's weight sets off the valve thus releasing the spray of jetting fluid from the nozzles onto each sheep. Approximately 2-4 L of jetting fluid is applied to each sheep at a pressure of 400-500 kPa. After application, the sheep are let out into a holding yard. The final concentration of diazinon in the jetting solution used was 0.2 g/L at one session and 0.4 g/L at the other two sessions.

Typical tasks carried out by applicators were:

Moving the sheep that block the race entry or exit
Maintaining pumps for jetting solution

The number of sheep treated per session and diazinon deposited on different body regions are shown in table 6.

Table 6: Diazinon deposition calculated for different body regions in volunteers treating sheep in auto race jetting

VOLUNTEERS	EXTERNAL PATCHES							INTERNAL PATCHES				
	Head (µg)	Feet (µg)	Hands (µg)	Total body excluding head, hands and feet (µg)	Total body (µg)	No. of sheep treated	Exposure per 50 sheep treated (µg)	Hands (µg)	Total body excluding head, hands and feet (µg)	Total body (µg)**	No. of sheep treated	Exposure per 50 sheep treated (µg)
1	16.38	3.28*	664.3	5489.0	6173.0	50	6173.0	7.62	428.0	751.1	50	751.1
2	104.1	3.28	5225.4	8150.0	13483.0	50	13483.0	2.13	20330.9	20648.5	50	20648.5
3	218.7	3.28	622.8	13738.0	14582.6	50	14582.6	0.49	231.5	547.5	50	547.5
4	192.8	3.28	637.4	9895.0	10728.9	50	10728.9	158.7	774.5	1248.7	50	1248.7
5	5.4	3.28	620.1	5737.2	6366.7	50	6366.7	25.3	62.6	403.4	50	403.4
6	216.2	3.28	1063.8	10027.5	11310.7	55	10282.5	9.26	153.2	477.9	55	434.4
7	581.2	17.4	6933.4	31380.6	38914.7	55	35377.0	25.7	62.6	403.8	55	367.1
8	20.8	3.28	51.8	446.6	522.6	55	475.1	3.52	260.0	579.0	55	526.4
9	176.5	3.28	976.1	2198.3	3353.7	55	3048.8	1.97	62.6	380.0	55	345.5
10	106.1	11.3	1513.6	2078.1	3709.2	55	3372.0	5.58	125.3	446.3	56	398.5
11	1305.5	11.79	836.0	20767.6	22921.0	50	22921.0	4.26	976.5	1296.2	50	1296.2
12	311.4	3.28	617.3	5187.7	6120.1	50	6120.1	3.28	195.1	513.8	50	513.8
13	144.1	9.82	2474.1	4422.1	7049.7	50	7049.7	0.49	103.7	419.7	50	419.7
14	764.4	8.50	2513.1	9821.7	13107.2	50	13107.2	9.43	62.6	387.5	50	387.5
15	473.8	3.28	1491.0	6806.7	8773.8	50	8773.8	0.49	115.0	431.0	50	431.0
Mean	**309.2**	**6.3**	**17493**	**9076.4**	**11141.0**		**10790.8**	**17.2**	**1596.3**	**1929.0**		**1914.6**

Head and feet exposures were not measured for 'Internal patches' group. Arithmetic means of the head and feet depositions obtained in the 'External Patches' group were added to all 'Internal Patches' group volunteers to calculate total body deposition in that group (see Section 3.3).

* Where the laboratory reported value was less than the limit of detection (1 µg/container or 10 ng/cm^2), a value of 5 ng/cm^2 was used as a default value. This value was multiplied by the respective body part area to obtain deposition on that area.

** (Total body excluding head, hands and feet) + hands + deposition on head and feet obtained in unprotected workers.

Comments

In the auto race jetting, detectable levels of diazinon were found on hands of 12 of the 15 volunteers wearing cotton gloves beneath the protective gloves. However, these exposures were very low indicating adequate protection from gloves (Table 6). Nearly half the volunteers in this group also had detectable diazinon contamination on forearms, shoulder and/or thigh (individual body part exposure data not shown). However, total body contamination in this group was high indicating that overalls provided just over 80% protection. Inhalation pads of 11 of the 15 volunteers were contaminated indicating that spray drift or splashes occurred during this treatment (data not shown). However, the inhalation exposure was very low; highest contamination being 12.3 μg for 50 sheep treated.

The highest and lowest total dermal exposure levels detected during the study for Auto Race jetting operators were 35.4 mg diazinon and 0.47 mg diazinon per 50 sheep treated respectively, for those not wearing PPE and 20.6 mg diazinon and 0.39 mg diazinon per 50 sheep treated respectively, for the those wearing PPE (Table 6).

2.4.5 Application by Fixed Plunge Dip

Fifteen replicates, each for protected and unprotected fixed plunge dipping were monitored at two sites. In this application method, sheep enter a plunge dip via a straight or curved race. There is approximately a 0.5 m vertical drop from the race into the dipping solution. A typical concrete plunge dip is 12 m long and 0.75 m wide. The sheep become submerged on entering the dip and swim through the dip to the other end. They are submerged twice more in the swim section by individual operators each using a dunking stick. The sheep then begin a 3-4.5 m, 45^{o} ascent out of the dip into the draining pen.

The dipping solution that has drained from the sheep re-enters the dip via a sump with a strainer to remove extraneous matter. The final concentration of diazinon in the dip solution used was 0.075 g/L at two sessions and 0.083 g/L at the third session.

Typical tasks carried out by applicators were:

Moving the sheep that block the race entry or exit
Dunking sheep

The number of sheep treated per session and diazinon deposited on different body regions are shown in table 7.

Table 7: Diazinon deposition calculated for different body regions in volunteers treating sheep by fixed plunge dip

VOLUNTEERS	EXTERNAL PATCHES							INTERNAL PATCHES				
	Head (µg)	Feet (µg)	Hands (µg)	Total body excluding head, hands and feet (µg)	Total body (µg)	No. of sheep treated	Exposure per 50 sheep treated (µg)	Hands (µg)	Total body excluding head, hands and feet (µg)	Total body (µg)**	No. of sheep treated	Exposure per 50 sheep treated (µg)
1	5.42	9.95	158.0	940.0	1114	57	977	1.64	62.6	124.9	57	109.6
2	46.2	3.28	31.2	708.3	789	57	692	0.49	62.6	123.7	57	108.5
3	88.9	3.28	61.4	1140.0	1294	57	1135	2.29	62.6	125.5	57	110.1
4	5.42	8.25	25.9	162.0	201	57	177	7.3	62.6	130.5	57	114.5
5	5.42	32.9	34.2	113.4	186	57	163	2.5	62.6	125.7	50	125.7
6	5.42	3.28	12.0	326.6	347	48	361	4.3	62.6	127.5	48	132.8
7	5.42	3.28	378.0	477.6	864	48	890	5.2	109.7	175.5	48	182.8
8	5.42	3.28	11.2	763.8	783	48	816	4.75	62.6	128.0	48	133.3
9	8.97	3.28	159.7	15126.6	15298	48	15935	3.8	62.6	127.0	48	132.3
10	5.42	3.28	35.5	2247.1	2292	48	2387	4.7	95.7	161.0	56	143.8
11	40.9	3.28	111.0	34644.1	34799	51	34117	1.9	62.6	125.1	51	122.6
12	44.7	11.8	170.2	19225.7	19451	51	19070	5.9	82.0	148.5	51	145.6
13	435.6	3.28	631.2	12706.2	13776	51	13506	11.7	331.7	404.0	51	396.1
14	58.8	7.60	1099.8	2241.0	3407	51	3340	13.8	62.6	137.0	51	134.3
15	44.2	3.28	36.2	756.0	840	51	823	2.4	73.3	136.3	51	133.6
Mean	**53.7**	**6.89**	**197.0**	**6789.2**	**6362.7**		**6292.6**	**4.84**	**87.9**	**153.4**		**148.4**

Head and feet exposures were not measured for 'Internal patches' group. Arithmetic means of the head and feet depositions obtained in the 'External Patches' group were added to all 'Internal Patches' group volunteers to calculate total body deposition in that group (see Section 3.3).

* Where the laboratory reported value was less than the limit of detection (1 µg/container or 10 ng/cm^2), a value of 5 ng/cm^2 was used as a default value. This value was multiplied by the respective body part area to obtain deposition on that area.

** (Total body excluding head, hands and feet) + hands + deposition on head and feet obtained in unprotected workers

Comments

In the fixed plunge dip component of the study, thigh, lower legs and hands were most contaminated in all external patch volunteers, followed by forearms and shoulders and neck/chest (Data not shown). In the internal patch group, all volunteers had detectable or quantifiable levels of diazinon on hands, albeit at very low levels (Table 7) and only 4 had contamination on other regions of the body, such as forearm, shoulder and lower leg (data not shown). Gloves and overalls provided more than 95% protection (Table 7). There was no exposure by the inhalation route.

The highest and lowest total dermal exposure levels detected during the study for fixed plunge dip operators were 34 mg and 0.16 mg diazinon per 50 sheep treated respectively, for those with external patches and 0.41 mg, and 0.12 mg diazinon per 50 sheep treated respectively, for workers wearing PPE (internal patches) (Table 7).

2.4.6 Application by Shower Dip

A typical shower dip facility consists of a cylindrical structure 4.5 m in diameter with a wall height of 1.8 m made of corrugated wrought iron sheets attached to galvanised steel frame. The structure is anchored to a concrete floor. From within the dip there is a spillway to allow the dipping fluid to drain back into an adjacent concrete sump with a capacity of 1250-2500 L.

Leading from the sump, a 100 mm diameter pipe that attaches to the wall of the dip and then reaches across and ends with a rotating spray 'arm' centred at about 1.75 m from the floor. The arm has a central nozzle and driving nozzles at each end that are set at about 35^{o} opposing angles. In addition, the 100 mm pipe connects to smaller 25 mm diameter pipes leading to spray nozzles on the floor of the dip. The nozzles are equivalent distance from each other and are arranged in a circular design starting around the wall with another 2 sets completing the set-up in the centre of the flooring.

A small pump is used to deliver the dipping solution to the pipes and nozzles. The operating pressure at the nozzles is maintained at 100 kPa and the spray arm rotates at 5 rotations per minute. Shower dips have a capacity of 50 to 60 adult sheep. Sheep enter the dip directly from the yard via an entry door and remain under the shower for about 12 minutes. The sheep are then allowed to drain for approximately 5 minutes before being released through an exit door. The dipping solution that has drained from the sheep re-enters the sump via a strainer to remove extraneous matter.

The product label (Jetdip) recommends a concentration of 10 mg/100 mL diazinon (0.01%) in the dip solution for body lice control and 20 mg/100 mL diazinon (0.02%) in the dip solution for blowfly control. Accordingly, two sessions of shower dip treatment used 0.01% diazinon solution and one session used 0.02% diazinon solution. It is not stated whether recharging the dip solution was required in any of the session although this is unlikely give the low number of animals treated in each session.

Typical tasks carried out by applicators were:

Redirecting the sheep along that block the race entry or exit
Connecting and disconnecting hoses.

The number of sheep treated per session and diazinon deposited on different body regions are shown in table 8.

Table 8: Diazinon deposition calculated for different body regions in volunteers treating sheep by shower dip

VOLUNTEERS	EXTERNAL PATCHES							INTERNAL PATCHES				
	Head (µg)	Feet (µg)	Hands (µg)	Total body excluding head, hands and feet (µg)	Total body (µg)	No. of sheep treated	Exposure per 50 sheep treated (µg)	Hands (µg)	Total body excluding head, hands and feet (µg)	Total body (µg)**	No. of sheep treated	Exposure per 50 sheep treated (µg)
1	5.42*	3.28*	164.5	153.1	326.3	50	326.3	1.89	62.6	168.0	45	186.7
2	5.42	3.28	21.4	81.9	112.0	45	124.4	3.61	1058.2	1165.3	45	1294.8
3	5.42	3.28	4.90	73.7	87.3	45	97.0	31.4	62.6	197.5	45	219.4
4	5.42	198.3	22.2	72.4	298.3	45	331.4	17.1	263.1	383.7	45	426.3
5	5.42	9.95	75.0	129.8	220.2	45	244.7	1.80	62.6	167.9	45	186.6
6	172.0	3.28	572.1	1152.6	1900.0	50	1900.0	2.79	111.0	217.3	50	217.3
7	85.3	3.28	319.3	752.0	1159.8	50	1159.8	1.56	62.6	167.6	50	167.6
8	260.9	3.28	566.2	2085.9	2916.3	50	2916.3	14.4	126.6	244.5	50	244.5
9	92.6	96.5	407.9	1029.1	1626.2	50	1626.2	0.49*	62.6	166.6	50	166.6
10	167.4	11.9	265.2	1347.1	1791.6	50	1791.6	14.2	235.4	353.1	45	392.3
11	55.6	3.28	28219.6	1094.1	29372.8	60	24477.3	9.27	215.3	328.1	60	273.4
12	130.3	8.90	1305.8	2457.6	3902.6	60	3252.2	28.1	69.1	200.7	60	167.3
13	92.9	3.28	3591.8	3436.2	7124.2	60	5936.8	2.95	90.4	196.8	60	164.0
14	76.8	6.70	3159.3	2868.0	6110.9	60	5092.5	11.2	1106.2	1220.8	60	1017.3
15	20.9	11.9	349.2	4531.4	4914.4	60	4095.3	8.04	94.5	206.0	60	171.7
Mean	**78.8**	**24.7**	**2603**	**1417.7**	**4124.2**		**3558.0**	**9.92**	**245.5**	**358.9**		**353.0**

Head and feet exposures were not measured for 'Internal patches' group. Arithmetic means of the head and feet depositions obtained in the 'External Patches' group were added to all 'Internal Patches' group volunteers to calculate total body deposition in that group (see Section 3.3).

* Where the laboratory reported value was less than the limit of detection (1 µg/container or 10 ng/cm^2), a value of 5 ng/cm^2 was used as a default value. This value was multiplied by the respective body part area to obtain deposition on that area.

** (Total body excluding head, hands and feet) + Hands + deposition on head and feet obtained in unprotected workers

Comments

During shower dip application, almost all volunteers with internal patches had hand and lower leg contamination albeit at very low level (Table 8) and 10/15 had shoulder, forearm and neck (front and back) contamination (data not shown). Comparison with exposure values in external patch group indicated good protection by gloves (>95%) but only 82.7% from overalls (Table 8). Feet were the least exposed. Inhalation exposure was negligible and did not contribute to total exposure (data not shown). The highest and lowest total dermal exposure levels detected during the study for shower dip operators were 24.5 mg and 0.1 mg diazinon per 50 sheep treated respectively, for those with external patches and 1.47 mg and 0.15 mg diazinon per 50 sheep treated respectively, for those with internal patches.

2.4.7 Cleanup

Typical tasks carried out by cleaners were:

Rinsing hoses and nozzles
Emptying out sumps and tanks onto adjacent grassed area
Hosing down utensils and equipment
Washing down contaminated areas.

The diazinon deposited on different body regions is shown in table 9.

Table 9: Diazinon deposition calculated for different body regions in volunteers cleaning up treatment areas and equipment

VOLUNTEERS	EXTERNAL PATCHES					INTERNAL PATCHES		
	Head (μg)	Feet (μg)	Hands (μg)	Total body excluding head, hands and feet (μg)	Total body (μg)	Hands (μg)	Total body excluding head, hands and feet (μg)	Total body (μg)**
1	21.7	7.99	6931.4	3218.5	10179.6	23.9	62.6	142.8
2	21.4	3.28*	960.0	1350.6	2335.3	1.48	92.8	150.6
3	136.1	3.28	17168.4	2771.8	20079.7	3.36	101.5	161.2
4	7.84	22.3	3593.1	200.5	3823.8	0.49	62.6	119.4
5	9.66	86.5	3201.8	9790.1	13088.0	8.69	197.5	262.5
6	5.42	3.28	3542.7	606.6	4157.8	10.4	62.6	129.3
7	5.42	3.28	13478.3	477.8	13964.7	0.49	62.6	119.4
8	39.6	19.1	7328.0	8484.9	15871.5	10.3	1124.3	1191.0
9	39.6	19.1	7328.0	8484.9	15871.5	4.92	83.8	145.1
10	22.1	3.28	500.8	1931.2	2457.3	3.11	62.6	122.1
11	73.4	6.81	4642.5	2954.4	7677.0	33.4	1031.5	1121.2
12	5.42	145.7	31283.5	2180.0	33614.5	1.40	2143.2	2200.9
13	5.42	110.7	428.6	71.2	615.9	1.23	74.8	132.4
14	5.42	3.28	238.9	152.6	400.2	0.49	62.6	119.4
15	5.42	3.28	284.5	62.6	355.8	8.04	150.3	214.7
Mean	**26.9**	**29.4**	**6727.4**	**2849.2**	**9632.8**	**7.45**	**358.3**	**422.1**

Head and feet exposures were not measured for 'Internal patches' group. Arithmetic means of the head and feet depositions obtained in the 'External Patches' group were added to all 'Internal Patches' group volunteers to calculate total body deposition in that group (see Section 3.3).

* Where the laboratory reported value was less than the limit of detection (1 μg/container or 10 ng/cm^2), a value of 5 ng/cm^2 was used as a default value. This value was multiplied by the respective body part area to obtain deposition on that area.

** (Total body excluding head, hands and feet) + hands + deposition on head and feet obtained in unprotected workers

Comments

As can be expected, hands and thighs had high levels of diazinon residues in those cleaning up after diazinon application (external patch group). Hand contamination was lowest during cleaning of the shower dip area/equipment compared with other application methods. In the internal patch group, 13 of the 15 protected volunteers had hand contamination (very low, Table 9), while detectable levels of diazinon on other body regions were noted only in 5 volunteers (data not shown). Gloves afforded almost complete protection to hands. However, overalls provided only ~86% protection to workers.

The highest and lowest total dermal exposure levels detected during the study for Clean up work were 58 mg and 0.50 mg per kg diazinon handled, respectively for unprotected workers and 3.8 mg and 0.13 mg per kg diazinon handled, respectively for the protected workers.

2.5 OHS risk assessment

The exposure data from these studies was used to complete a quantitative risk assessment to workers using diazinon products for sheep treatment. Risk to workers during mixing/loading and treating sheep by each of the following commonly used application methods was assessed:

Portable Plunge Dipping
Hand Jetting
Auto Race Jetting
Fixed Plunge Dipping
Shower Dipping

Diazinon product was applied in accordance with label specifications, varying from 0.1 g diazinon/L (plunge and shower dips for lice control) and 0.2 g diazinon /L (plunge and shower dips for blowfly control) to 0.4 g diazinon/L (jetting for blowfly control). Table 10 shows the application rates of diazinon recommended on product labels.

Table – 10: Use pattern of diazinon products in sheep using various application methods

Application Method	Pest	Formulation type and concentration of ai in product	Application rate /dilution of product (concentration of ai in solution)
Plunge dip	Lice, Ked, Blowfly, Itchmite	EC 200 g/L, EC 80 g/L, EC 60 g/L	Initial charge - 0.01% -0.02% ai Reinforcing 650 mL – 1.2 L of undiluted product when dip level falls by 500 L Topping up - (0.01% -0.03% ai
Conventional shower dip	Lice, Blowfly Itchmite	EC 200 g/L, EC 80 g/L, EC 60 g/L	Initial charge (0.01% - 0.02% ai) Reinforcing 250 mL – 500 mL of undiluted product when dip level falls by 200 L Topping up 250 mL- 500 mL per 500 L water (0.01% - 0.02% ai)
Spray race	Lice, Ked	EC 200 g/L	500 mL per 1000 L water (0.01% ai)

Automatic jetting	Blowfly	EC 200 g/L	400 mL per 200 L water (0.04%)
Hand jetting	Blowfly	EC 200 g/L	400 mL per 200 L water (0.04% ai)
Backline long wool treatment	Lice, Blowfly	EC 96 g/L	5.25 mL – 10.5 mL undiluted product per sheep (9.6% ai) Application volume depends on pest and length of wool (higher rate for longer wool)
Backline off shears treatment	Lice	EC 93.3 g/L	1 part of product to 6 parts of water (0.15% ai) Apply approximately 3 mL per kg live weight
Wound dressing		EC 1 g/L, EC 3 g/L, EC 200 g/L, PD 15 g/kg, PD 20 g/kg	20 mL undiluted product per wound (0.06 - 0.1% ai)

2.5.1 Occupational exposure

The study authors measured the time spent by each subject treating approximately 50 sheep (5 volunteers per session) and estimated the total amount of diazinon applied during a session. The amount of diazinon used by each worker was then calculated as a function of time spent treating ~50 sheep. Exposure to diazinon was expressed as micrograms diazinon deposited on the body per kg of diazinon used per hour.

For plunge and shower dipping the accuracy of expressing exposure as a function of diazinon used in carrying out the operation is questionable since the 'applicators' do not actually handle the products, rather they only direct the sheep through the dip solution or under the shower nozzles. Similarly, for hand and auto race jetting, the exposure of workers cannot readily be expressed as a function of the speed at which sheep are treated or the volume of diazinon handled as these parameters are too variable.

For the reasons indicated, occupational exposure of the workers during application of product is now expressed as micrograms of diazinon per 50 sheep treated. This value was extrapolated to give exposures resulting from treatment of 500 and 2000 sheep in order to estimate the daily exposure of sheep handlers using hand/race jetting and plunge/shower dipping methods, respectively (based on previous data indicating that in one day approximately 500 sheep can be treated by jetting and 2000 sheep can be treated by dipping, NOHSC 1999).

For mixer/loaders the volume of diazinon solution used up following treatment of approximately 250 sheep by each treatment method was provided. These data were used to calculate exposure for mixer/loaders. Thus for the OHS risk assessment exposure for mixer/loaders was expressed as the amount of diazinon deposited on the body when diazinon solution enough to treat a minimum of 250 sheep was prepared.

Exposure values within each treatment group varied considerably, reflecting different working habits of workers. Arithmetic mean of the fifteen observations for each application method was used for calculating the risk to workers. The reason for using an arithmetic mean was that successive daily exposure would be anticipated to be quite variable and not subject to continuous high or low levels. An arithmetic mean gives weight to the very high and very low exposures according to their probability of occurrence. The individual body weights of the volunteers were not provided in the

study. Exposure values were divided by a default body weight value of 70 kg to obtain exposure per kg body weight. Inhalation exposure during mixing/loading and application was observed to be negligible and therefore the contribution of this component of the exposure was not included in the overall risk assessment.

2.5.2 Selection of NOEL and dermal absorption

In order to perform an occupational risk assessment careful consideration needs to be given to the selection of the appropriate toxicological endpoint and duration of dosing. An inspection of all repeat-dosing studies in the database for diazinon indicated that the most sensitive endpoint by all routes of administration, durations and species was cholinesterase inhibition. This endpoint is also considered relevant for an occupational risk assessment. As humans are the species which are being protected it is appropriate that human data, of suitable quality and availability, is used for risk assessment purposes. The dietary risk assessment for diazinon is based on the inhibition of plasma cholinesterase in a 37-43 day oral dosing study in humans (NOEL = 0.02 mg/kg bw/day for plasma ChE inhibition) (APVMA Diazinon Review, 2002). Although a 37-43 day repeat dosing study may be considered to be too short to ensure life-long dietary protection it is the consistency of the endpoint in relation to the dosing duration and its occurrence in all species, which is important. Consequently the same study in humans can be used as the basis of the occupational risk assessment.

However, since the volunteers in the human study experienced exposure via ingestion of diazinon a dermal absorption factor needs to be applied to make the risk assessment relevant for the major occupational route of exposure for this assessment, ie. dermal. A study conducted in human volunteers is the most relevant dermal absorption study. Considering the variability of the dermal absorption rates in the study a value of 4% will be used in the OHS risk assessment (Wester et al., 1993). Since diazinon, like most organophosphorus pesticides, is almost completely absorbed from the gastrointestinal tract no correction is required to the NOEL for the purpose of determining the predicted ‘internal’ dose.

Although it is usual for the acceptable MOE to be 10 for an occupational risk assessment when the NOEL is derived from a human study, a factor of 20 has been selected in the case of diazinon. The additional 2-fold safety factor has been applied due to the closeness of the NOEL and the LOEL (0.025 mg/kg bw/day) and the limited nature of the study (APVMA Diazinon Review, 2002).

The calculated MOE for volunteers involved in treating sheep are shown in Table 11.

Table 11 – Dermal exposure and MOE during sheep treatment with diazinon products

Application Method	Total Dermal Exposure (µg diazinon) per 50 sheep	Total Dermal Exposure (µg diazinon) per 500 sheep[a] per 2000 sheep[b]	Dermal absorbed dose (mg diazinon/kg bw)	MOE	No. of sheep that can be treated to achieve a MOE of 20
Mixing/Loading – without PPE	78882*	78882*	0.045	0.50	6
Mixing/Loading - with PPE	94.6*	94.6*	0.00005	370	4625
Portable Plunge Dip - without PPE	6804	272160[b]	0.155	0.13	13
Port. Plunge Dip – with PPE	407.5	16300[b]	0.009	2	215
Hand Jetting - without PPE	136987	1369870[a]	0.782	0.026	1
Hand Jetting - with PPE	95981	959810[a]	0.548	0.036	1
Auto Race Jetting - without PPE	10791	107910[a]	0.062	0.32	8
Auto Race Jetting - with PPE	1914.6	19146[a]	0.011	2	46
Fixed Plunge Dip - without PPE	6293	251720[b]	0.144	0.14	14
Fixed Plunge Dip - with PPE	148.4	5936[b]	0.0034	6	590
Shower Dip - without PPE	3558	142320[b]	0.081	0.25	25
Shower Dip - with PPE	353	14120[b]	0.0081	2.5	248
Clean up - without PPE	9633**	9633**	0.0055	3.6	-
Clean up - with PPE	422**	422**	0.00024	83	-

*µg diazinon/volume of diazinon solution prepared to treat 250 sheep

**µg diazinon. This is independent of the number of sheep treated

Dermal absorbed dose (mg/kg bw/day) = Total dermal exposure (per amount of diazinon mixed/loaded for 250 sheep or per 500/ 2000 sheep treated) x 4% dermal absorption ÷ 70 kg bw ÷ 1000 (mg)

MOE = NOEL (0.02 mg/kg bw/day) ÷ Total absorbed dose (mg/kg bw/day).

2.5.3 Margin of Exposure

The study results showed that MOEs for workers without PPE were very low for all treatment methods (including mixing/loading and cleaning) indicating the need for PPE when using diazinon for sheep treatment by any method.

For workers wearing PPE, the MOE was acceptable (>20) for mixer/loaders (Table 11) and workers cleaning the treatment areas and equipment after sheep treatment. Since exposure to diazinon during mixing/loading was expressed as micrograms (µg) diazinon deposited following preparation of diazinon solution to treat a minimum of 250 sheep, a MOE of 370 for mixer/loaders indicates that diazinon solution for treating up to 3425 sheep could be prepared per day without posing an unacceptable exposure to workers.

As indicated in Section 5.1, the risk to workers treating sheep was calculated on the basis of the number of sheep a farmer or contractor would normally treat per day (500 or 2000 per day, depending on treatment method). Total body deposition of diazinon obtained in the study from treating approximately 50 sheep was extrapolated to give exposure resulting from treating 500 or 2000 sheep (Table 11). This conclusion is valid on the assumption that exposure to diazinon is linearly proportional to the number of sheep treated.

Results showed that the MOE for **ALL** treatment methods was unacceptable, even when workers wore the label-specified PPE. Exposure during hand jetting was highest followed in order by auto race jetting, portable plunge and shower dipping and fixed plunge dipping. The results indicated an unacceptable exposure to workers using diazinon products for treating sheep by all treatment methods.

It should be also be noted that the calculated MOEs assume that different individuals are responsible for each task, ie mixing/loading, application and clean up after treatment has finished.

2.6 Discussion and conclusion

The study measured worker exposure to diazinon during mixing/loading and sheep treatment by each of the five application methods commonly used in Australia. Exposure was measured with and without the label recommended PPE. Each application method was carried out 3 times in three separate sessions at two or three different sites. It was noted that although different concentrations of diazinon were used in the three sessions for a particular application method, (for example, in the plunge dipping method, 0.01% diazinon was used in one session and 0.02% diazinon was used in the other two sessions), worker exposure values were not significantly different in these sessions. Large variation in exposure values within a group may have precluded or masked any actual difference in exposure from 0.01% and 0.02% diazinon solutions.

The study report suggested that, for all application methods, and where necessary, recharging of diazinon solution was conducted by animal handlers and where indicated, by applicators. However, no further details of the method of re-charging the solution, amount of diazinon required to re-charge and any exposure from this task were provided. This may be because no recharging was required given the experimental design.

There were also no details provided regarding laboratory controls (eg. interassay variability) or recovery reproducibility for the GC analysis of the samples.

Inhalation exposure was measured using chromatographic paper patches placed in the respirators. The results showed that in all application methods inhalation exposure was negligible when compared to dermal exposure. Exposure via inhalation was therefore not included in the overall risk assessment.

The MOE was unacceptable for workers using diazinon to treat sheep by dipping or jetting procedures indicating that the label prescribed PPE did not provide adequate protection (Table 11).

In all treatment methods gloves were effective in blocking >95% diazinon residue from coming in contact with the hands. Cotton overalls afforded >95% protection to workers only during plunge dipping. For other treatment methods, more than 20% of the total diazinon detected by the external patches penetrated the cotton overalls.

Worker contamination was highest during hand jetting, with overalls providing only about 25% protection. This resulted in the MOE being less than 1 even with PPE (Table 11). Thighs and lower legs had high diazinon residues, suggesting extensive splashing during jetting application. Indirect measurements indicated head and face contamination were high during hand and auto race jetting; most likely from splashing. If the use of products containing diazinon on sheep is to continue additional consideration will be given to whether head and face protection, such as that provided by a faceshield, should be employed when using diazinon products for sheep treatment.

The mixing/loading of diazinon gave an acceptable MOE (MOE=370) when workers wore PPE. From a consideration of the MOE a worker could safely mix and load sufficient diazinon to treat up to 4600 sheep.

For applicators the number of sheep that could be treated to achieve an acceptable MOE (MOE=20) was calculated for each treatment method. Results indicated that only around 200 sheep could be treated per day by portable plunge or shower dipping. This is almost one-tenth of the average number of sheep normally treated in one day by these methods. For hand and auto race jetting also, the number of sheep that could be treated per day in order to achieve an acceptable MOE were very small (2 and 45, respectively). Treatment of such small numbers of animals over several days, especially on large farms where their numbers could exceed a thousand, is not practical and so these modes of application can no longer be supported.

Although the OHS risk assessment for applicators has assigned 50% of the LOD for each patch non-detect, a recalculation of the MOE assuming a true zero value for the non-detect results would not raise the MOE to an acceptable level for any method of application.

Similarly, exclusion of the very high exposure values in each of the treatment groups (eg. 20,648 micrograms for total body exposure in auto race jetting) would not result in an acceptable MOE for any method of application.

However, it does appear possible to increase the number of workers involved in treating sheep so that each individual would treat no more in a day than the limits specified by this analysis, as detailed above. However, this proposal would only be viable for some application methods ie. plunge or shower dipping. For hand and auto race jetting the number of workers needed would be unrealistic. In addition, compliance with and the practicalities of such use restrictions are questionable.

Another possibility to reduce exposure might be to wear an extra layer of waterproofing. A PVC or rubber apron over waterproof overalls could be expected to provide extra protection during treatment. However, the practicality of wearing such PPE all day long in hot weather is also questionable. In addition, at present there are no data available to estimate the extent of protection such additional PPE can provide.

From the limitations associated with all these options it becomes apparent that new, safer ways of treating sheep with organophosphorus pesticides need to be considered.

It is concluded from the results of this study that the currently recommended PPE do not provide adequate protection to workers when using diazinon products for sheep treatment in accordance with current label recommendations.

2.7 Recommendations to the APVMA

1. The OCS recommends that the APVMA NOT be satisfied that use of diazinon products for treating sheep by plunge/shower dipping or hand/auto race jetting methods will not cause adverse effects on the health and safety of persons using such treatment methods.

2. The APVMA may wish to consider the following options to reduce potential occupational exposure:

    a) Use of waterproof PPE.
    b) Increase the number of workers involved in carrying out treatments.
    c) Consider alternate sheep handling methods.

2.8 References

APVMA Diazinon Review, (2002).
http://www.apvma.gov.au/chemrev/diazinontox.pdf

National Occupational Health and Safety Commission (1999). Report of the field visit to Elizabeth Macarthur Agricultural Institute, NSW. 17 March 1999.

Wester RC, Sedik L, Melendres J, Logan F, Maibach HI & Russel I (1993) percutaneous absorption of diazinon in humans. Food Chem Toxicol 31**:** 569-572.

2 RESIDUES

3.1 Introduction

3.1.1 Background

The Chemistry and Residues Program (CRP) prepared a draft copy of the Residues Report for the APVMA Review of Diazinon, and this report was submitted to the (then) NRA Chemical Review Section in June 2000. Subsequently, the APVMA published notices in the Australian rural and metropolitan press, calling for written submissions regarding the review of the chemical diazinon.

During the public consultation phase of the review, a number of grower groups/stakeholders indicated their support for the continued registration of diazinon use patterns in bananas, beetroot, cauliflower, mushrooms, onions, pineapple and rhubarb. Advice on the number of residue trials required to enable the promulgation of permanent MRLs for these commodities was provided to both the Stakeholders and to the APVMA Review Section on 24 September 2001.

Confirmatory Australian residue data have been provided to support the continued registration of diazinon in ***bananas***, ***bulb onions, pineapples and mushrooms***. These data are evaluated in this report.

3.1.2 Recommendations from Previous Draft Review Reports

The draft review report for diazinon was published in September 2002, in which a number of recommendations for MRL amendments were made. Those recommendations included amendments to the existing MRLs and the inclusion of new MRLs for edible offal (mammalian), milks, bananas, beans, blueberries, brassica vegetables, bulb vegetables, cereal grains, citrus fruits, cucurbits, fruiting vegetables, grapes, hops, leafy vegetables, peas, pineapple, pome fruit, root and tuber vegetables, stalk and stem vegetables, stone fruits, sugar cane, tree nuts and vegetable oils. Since the report was published, further data have been received and assessed and new recommendations in relation to MRL amendments are made in this report, which supercede previous MRL recommendations.

3.1.3 Overview of the Data Package

In support of the proposals to retain the registered uses of diazinon in bananas, bulb onions, pineapples and mushrooms, the following studies were submitted, and are reported in Appendix 1.

Reference No.	Title
A1.1	***Trials to determine the level of diazinon in bananas following two applications as a butt spray.*** (2002) Report No. 1/VX01029. Protocol No. ECR-DZN. Study Director: J. Clark, QFVG, Rocklea, QLD. Author: K. Bodnaruk, West Pymble, NSW. Analytical Laboratory: Analytical Services, Natural Resource Sciences Laboratories, Indooroopilly, QLD.
A1.2	***Trials to determine the level of diazinon in mushrooms at harvest following application to the casing layer.*** (2004) Report No.: AMGA/01/04. Study Director: A. Clift, University of Sydney, NSW. Author: K. Bodnaruk, West Pymble, NSW. Test Facility: Agrisearch Analytical Pty Limited, Rozelle, NSW.

A1.3	***Trials to determine the level of diazinon in onions at harvest following two applications as a butt spray (sic).*** (2002) Report No. VX01029. Protocol No. ECR-002. Study Director: K. Bodnaruk, West Pymble NSW. Author: K. Bodnaruk, West Pymble NSW. Analytical Laboratory: Victorian State Chemistry Laboratory, Werribee, VIC.
A1.4	***Submission of residue field trial data for determination of a NRA MRL in pineapples for diazinon.*** (2003) Sponsor: A&C Rural, Narangba, QLD. Author: D. Corbett, A&C Rural Pty Limited. Analytical Laboratory: Agrisearch Analytical Pty Limited, Rozelle, NSW.

3.2 Residues trials

3.2.1 Bananas

The Australian banana growers indicated in their submission that the industry preferred to retain the use of diazinon as a butt spray with a maximum of two applications of 100 g diazinon/100 L per season, applied at 14 day intervals; equivalent to 0.6 g diazinon/pseudostem base; nil harvest WHP.

Details of 4 Australian residue trials conducted in bananas are provided in Appendix 1, and the results are summarised below.

Diazinon residues in bananas that were treated with two butt spray applications of 0.6 g diazinon/plant at 14 day intervals (ie 1× the maximum proposed rate) are tabulated below. Residue values have not been corrected for method recoveries.

Trial Number	Trial location	Treatment regimen	Sampling time (DALT)	Diazinon residues (mg/kg)
1	Euramo QLD	Untreated control	0	<0.02
		2 applications of 0.6 g ai/plant, applied at 14 day intervals (1×)	0	<0.02
2	Upper Murray QLD	Untreated control	0	<0.02
		2 applications of 0.6 g ai/plant, applied at 14 day intervals (1×)	0	<0.02
3	Tully Valley QLD	Untreated control	0	<0.02
		2 applications of 0.6 g ai/plant, applied at 14 day intervals (1×)	0	<0.02
4	Feluga QLD	Untreated control	0	<0.02
		2 applications of 0.6 g ai/plant, applied at 14 day intervals (1×)	0	<0.02

When bananas are treated with 2 butt spray applications of 0.6 g diazinon/plant at 14 day intervals (ie 1× the maximum proposed rate), residues in bananas immediately after the second treatment were all below the limit of reporting (ie <0.02 mg/kg).

The 1993 JMPR reviewed a limited amount of data for the use of diazinon on bananas. In trials conducted in Honduras, banana plants received three sprays at 90 g ai/100L of a diazinon EC formulation. Banana pulp and peel were sampled at 0, 3, 7 and 14 days after the final application and diazinon residues were <0.02 mg/kg at all sampling intervals. The JMPR was unable to recommend a Codex MRL due to the limited data set.

The Australian data tabulated above, together with the limited data reported by the 1993 JMPR are considered sufficient to allow the establishment of a diazinon MRL of *0.02 mg/kg for bananas.

3.2.2 Mushrooms

There are currently two registered use-patterns for diazinon in mushrooms: (i) treatment of compost at spawning with 112 g diazinon/10L water/tonne moist compost, and (ii) treatment of mushroom casing with 24 g diazinon/10L water/tonne casing mixture, applied during preparation of the casing material, or as a spray over the top of the casing soil immediately after casing. The withholding period for this use is 14 days.

Cultivated mushrooms are usually grown in climate-controlled rooms. The fungal inoculum or "spawn" is added to a pasteurised substrate in growing containers or beds. After the fungal strands

(mycelia) have spread throughout the compost, a layer of peat or soil (the "casing") is added. The casing protects the compost from drying out, and allows for formation of the fruiting bodies (mushrooms). The mushrooms begin appearing about 6 weeks after spawning, and continue appearing in flushes about 7-10 days apart for a further 6-8 weeks. The first three flushes are the most productive. The cap and a small section of connected stem are usually harvested before the caps are fully expanded.

The Australian Mushroom Growers Association (AMGA) indicated that they wish to ***retain the use of diazinon in mushroom casing post-spawning***, and have provided confirmatory Australian residues data addressing the desired use-pattern. Full details of seven residue trials are provided in Appendix 1, and the results are summarised below.

Diazinon residues in mushrooms grown in casing that had been treated with a single application of 3.2 g diazinon/m^2 (equivalent to 24 g diazinon/tonne of casing mix) are summarized below. Residues have not been corrected for method recoveries (LOD = 0.01 mg/kg; LOQ = 0.05 mg/kg).

Treatment Regimen	Sampling time (DAT)	Diazinon residues (mg/kg)
Untreated control	--	<0.01
Mushroom casing treated with 3.2 g diazinon/m^2.	16-19 (first flush) 21-44	<0.01 to <0.05 <0.01

(i) Treatment of compost at spawning: No residues data have been provided in support of the use of diazinon in mushroom compost at spawning. Therefore, from a residues perspective, continued registration of this use pattern is ***not supported***.

(ii) Treatment of mushroom casing: The residues trials provided with the current submission involved treatment of mushroom casing with 24 g diazinon/10L water/tonne casing mixture, applied during preparation of the casing material (equivalent to 3.2 g diazinon/m^2 of casing). The results show that residues in the first flush of mushrooms (~3 weeks post-treatment) are all below the LOQ of the method (<0.05 mg/kg). Based on these data, an MRL of *0.05 mg/kg is considered appropriate to cover the occurrence of diazinon residues in mushrooms grown in diazinon-treated casing. A harvest WHP statement of "Not required when used as directed" is recommended for mushrooms, as there is a period of approximately 3 weeks between application of diazinon and the earliest time at which mushrooms are harvested.

3.2.3 Onions

Details of 3 Australian residue trials conducted in onions (bulb) are provided in Appendix 1, and the results are summarised below.

Diazinon residues in bulb onions that were treated with three foliar applications of 560 g diazinon/ha at 10 day intervals (ie 1× the maximum proposed rate) are tabulated below. Residue values have not been corrected for method recoveries.

Trial Number	Trial location	Treatment regimen	Sampling time (DALT)	Diazinon residues (mg/kg)
1	Narranderra NSW	Untreated control	3	0.057
		3 applications of 560 g ai/ha, applied at 10 day intervals (1×)	3 14	0.073 0.036
2	Wanneroo, WA	Untreated control	0	<0.01
		3 applications of 560 g ai/ha, applied at 10 day intervals (1×)	0 14	<0.01 <0.01
3	Forth, TAS	Untreated control	0	0.032

		3 applications of 560 g ai/ha, applied at 10 day intervals (1×)	0 14	0.032 0.024

When bulb onions are treated with 3 foliar applications of 560 g diazinon/ha at 10 day intervals (ie at the maximum 1× registered use rate), residues in onions at 14 DALT ranged from <0.01 mg/kg to 0.036 mg/kg. Based on these data, an MRL of 0.05 mg/kg is considered appropriate to cover the occurrence of diazinon residues in treated onions.

Data reviewed by the 1993 JMPR involved US trials where a pre-plant application at 4.4 kg ai/ha was followed by three foliar sprays at 0.55 kg ai/ha with either an EC or WP formulation. At 10 to 11 days after the final spray, diazinon residues were <0.01 to 0.04 mg/kg. At 14 days, residues in onions ranged from <0.01 – 0.02 mg/kg. The JMPR recommended an MRL of 0.05 mg/kg for onions on the basis of the data provided.

The Australian data together with the data reported by the JMPR, support the establishment of a diazinon MRL of 0.05 mg/kg for bulb onions.

3.2.4 Pineapples

The Australian pineapple growers indicated in their submission that they wish to retain the use of diazinon on pineapples. The currently registered use-pattern involves the application of up to 2.4 kg diazinon/ha at 2-3 week intervals as necessary (with no maximum number of applications specified), and a 14 day harvest WHP.

A&C Rural Pty Limited and Makhteshim Agan (Australia) Pty Limited provided confirmatory Australian residue data addressing the use pattern specified for pineapples on the product label for *Country Diazinon 800 Insecticide* (P41698). Details of 3 Australian residue trials conducted in pineapples are provided in Appendix 1, and the results are summarised below.

Diazinon residues in pineapples that were treated with foliar applications at a rate of 2.4 kg diazinon/ha are tabulated below. Residue values have not been corrected for method recoveries. (LOD = 0.005 mg/kg; LOQ = 0.01 mg/kg).

Trial Number	Trial location	Treatment regimen	Sampling time (DALT)	Diazinon residues (mg/kg)
1	Palmwoods, SE QLD	Untreated control	7/14†	<0.005
		26 applications of 2.4kg ai/ha, applied at 14 day intervals (1×)	3	0.07
			7	0.03
			14	0.04
			21	<0.01
2	Yeppoon, Central QLD	Untreated control	7/14†	<0.005
		2 applications of 2.4 kg ai/ha, applied at 7 day interval (1×)	7	0.01
			14	≤0.005
3	Glasshouse Mountains, QLD	Untreated control	7/14†	<0.005
		2 applications of 2.4 kg ai/ha, applied at 7 day interval (1×)	7	0.03
			14	≤0.01

†Untreated control samples were collected at 7 and 14 DALT. However, details of which of the control samples were analysed were not provided.

The trial conducted at Palmwoods (SE QLD) addressed the maximum registered (1×) use pattern. Pineapple plants were treated with 2.4 kg diazinon/ha at 14 day intervals, for a period of one year (ie 26 applications). A diazinon residue of 0.04 mg/kg was reported in pineapple at 14 days after the last treatment.

In the trials conducted at Yeppoon and the Glasshouse Mountains, pineapples were treated twice with 2.4 kg diazinon/ha at a 7 day interval. Given that diazinon is known to break down rapidly

in the environment (ie there is no evidence that diazinon residues accumulate as a result of multiple applications), retreatment of pineapples after a 7 day interval was considered appropriate to simulate the worst case residues scenario. Diazinon residues of <0.005 mg/kg and <0.01 mg/kg were reported in pineapple at 14 days after the last treatment.

Based on the confirmatory data provided, an MRL of 0.05 mg/kg is considered appropriate to cover the occurrence of diazinon residues in pineapples after treatment with an unlimited number of applications of 2.4 kg diazinon/ha at 14 day intervals, with 14 day harvest withholding period.

3.3 Dietary exposure estimates

3.3.1 Chronic Dietary Exposure

The chronic dietary risk is estimated by the National Estimated Daily Intake (NEDI) calculation, which is based on the mean consumption of relevant commodities by Australian consumers aged 2 years and above. The NEDI is then reconciled against the Acceptable Daily Intake (ADI) of 0.001 mg/kg bodyweight. The NEDI for diazinon was calculated to be 31.1 % of the ADI (see Appendix 2). As it is widely recognised that the NEDI calculation over-estimates the actual dietary intake, the risk to human health from the consumption of diazinon residues is considered to be acceptable.

3.3.2 Acute Dietary Exposure

The acute dietary risk is estimated by the National Estimated Short-Term Intake (NESTI) calculation, which is based on the 97.5th percentile consumption figures for relevant Australian consumers. The NESTI is then reconciled with the Acute Reference Dose (ARfD) for diazinon of 0.01 mg/kg body weight. The results of the NESTI calculations are tabulated below (see Appendix 2 for full details).

Commodity	Children (2-6 years of age)		Children and adults (2 years and over)	
	JMPR Scenario	NESTI Calculation (% of the ARfD)	JMPR Scenario	NESTI Calculation (% of the ARfD)
Bananas	Case 2a	4.6	Case 2a	1.3
Mushrooms	Case 1	1.4	Case 1	0.8
Onions (bulb)	Case 2b	3.6	Case 2a	1.7
Pineapple	Case 2b	42.8	Case 2b	14.1

The JMPR "Case 1" scenario is used when residues in the composite sample reflect residue levels in a meal sized portion, and the unit weight is <25 grams. The "Case 2a" scenario is used when composite residues data do not reflect residues in a meal-sized portion, and the unit weight is >25 grams and smaller than the large portion size. The "Case 2b" scenario is used when composite residue data do not reflect residues in a meal-sized portion, and the unit weight is >25 grams and larger than the large portion size. The NESTIs for bananas, mushrooms, onions and pineapples do not exceed 45 % of the ARfD in either of the scenarios examined. It is concluded that the acute dietary exposure to diazinon residues, as a result of consuming these treated commodities, is low and the risk is acceptable.

3.4 Residues in trade

The following MRLs have been established in major trading markets for meat, milk and offal products:

Commodity	Diazinon MRLs (mg/kg)					
	AUS	CODEX	USA	EU	Japan✦	Korea
Meat [in the fat]	0.7	2				0.7
Cattle fat			0.7		2	
Pig fat					2	
Sheep meat (fat basis)			0.7			
Other fat④						
Fat				0.7		
Muscle				0.02	0.02	
Milks	0.02	0.02	–	0.02	0.02	0.02
Edible offal	0.03	–	–	–	–	–
Kidney①	–	0.03	–	0.02	0.03	–
Liver②	–	0.03	–	0.02	0.03	–
Other offal③	–	–	0.7	–	0.7	0.02
Sheep meat by-products	–	–	0.7	–	–	–

✦ Japanese Provisional List 2006
① Kidney of cattle, goats, pigs & sheep
② Liver of cattle, goats, pigs & sheep
③ Other terrestrial mammals, edible offal (Jpn); cattle by-product (Korea)
④ Other terrestrial mammals, fat

In relation to meat, fat, milk and edible offal, the proposed Australian MRLs are either comparable to or lower than MRLs in major markets. It should be noted however that there is no milk entry listed in the US tolerances for diazinon.

For the purposes of a trade assessment, bananas, mushrooms, onions and pineapple are not listed in Appendix 1 of Part 5B of Ag MoRaG and therefore are not considered by APVMA to be commodities for which a trade assessment is required. However information sourced from the Australian Horticultural Statistics Handbook 2004, suggests that production of bananas, mushrooms and pineapple is mostly limited to domestic consumption (fresh and processed) with minor export volumes. Onions however are exported and major markets include the EU (Netherlands, U.K., France, Italy, Germany, Sweden) and Japan. Relevant onion MRLs are shown in the table below:

Commodity	Diazinon MRLs (mg/kg)			
	AUS	CODEX	EU	Japan✦
Onion	0.05	0.05	0.02	0.05

✦ Japanese Provisional List 2006

Comments on the trade aspects of diazinon residues in meat, milk, offal and onions are requested as part of the public consultation phase of the review.

3.5 Recommendations

The Chemistry and Residues Program (CRP) has considered residues data provided for bananas, mushrooms, onions and pineapple and recommended appropriate MRLs and withholding periods associated with the use of diazinon.

The following recommendations are made in relation to the continued use of diazinon in bananas, mushrooms, onions and pineapples:

1. It is recommended that registration of the use of diazinon in mushroom compost at spawning be cancelled, and references to this use-pattern should be removed from product labels.

2. The following use patterns are to be included on revised product labels:

Crop	Use Pattern
Bananas	Apply as a butt spray to the pseudostem. Maximum of two applications per season at 100 g ai/100L.
Onions	Apply at 560 g ai/ha, with a maximum of three applications per crop; apply at 10 day intervals.
Mushrooms	Apply 24 g ai/10L water/tonne of casing mixture; equivalent to 3.2 g ai/m^2 of casing. Apply during the preparation of the casing material.
Pineapples	Apply up to 2.4 kg ai/ha at 2 to 3 week intervals as necessary.

3. The following withholding period statements are recommended for inclusion on product labels:

   Bananas: Not Required When Used As Directed
   Mushrooms: Not Required When Used As Directed
   Onions: Do Not Harvest For 14 Days After Application
   Pineapples: Do Not Harvest For 14 Days After Application

4. The following amendments are proposed to Table 1 of the MRL Standard, to be implemented at various stages of proposed regulatory action:

Table 1

Code	Commodity	Current MRL	Proposed MRLs (phase-out period)	Proposed MRLs (post phase-out period)
FI 0327	Banana	–	–	*0.02
GC 0080	Cereal grains	0.1	T0.1	–
FC 0001	Citrus fruits	0.7	T0.7	–
MO 0105	Edible offal (mammalian)	0.7	T0.7	0.03
PE 0112	Eggs	*0.05	T*0.05	–
	Fruits (except citrus fruits, grapes, olives peach)	0.5	T0.5	–
FB 0269	Grapes①	T2	T2	–
FI 0341	Kiwifruit	0.5	T0.5	–

MM 0095	Meat (mammalian)[in the fat]	0.7	0.7	0.7
ML 0106	Milks [in the fat]	0.5	T0.5	
ML 0106	Milks	–	–	0.02
VO 0450	Mushrooms	–	–	*0.05
OC 0305	Olive oil, crude	2	T2	–
VA 0385	Onion, bulb	–	–	0.05
HH 0740	Parsley	T0.7	T0.7	T0.7
FS 0247	Peach	0.7	T0.7	–
FI 0353	Pineapple	–	–	0.05
PO 0111	Poultry, edible offal of	*0.05	T*0.05	–
PM 0110	Poultry meat	*0.05	T*0.05	–
VA 0388	Shallot	T0.5	T0.5	T0.5
VA 0389	Spring onion	T0.5	T0.5	T0.5
GS 0659	Sugar cane	0.5	T0.5	–
VO 0447	Sweet corn (corn on the cob)	0.7	T0.7	–
TN 0085	Tree nuts	0.1	T0.1	–
OC 0172	Vegetable oils (except olive oil, crude)	0.1	T0.1	–
	Vegetables	0.7	T0.7	–

①PER 4000 Expired

3.6 Appendix 1: Residue trial data

Reference

Trials to determine the level of diazinon in bananas following two applications as a butt spray. (2002) Report No. 1/VX01029. Protocol No. ECR-DZN. Study Director: J. Clark, QFVG, Rocklea, QLD. Author: K. Bodnaruk, West Pymble, NSW. Analytical Laboratory: Analytical Services, Natural Resource Sciences Laboratories, Indooroopilly, QLD.

Experiment

Four confirmatory Australian residues trials were conducted with diazinon in bananas. Details of each of the trials are tabulated below:

Details	Site 1	Site 2	Site 3	Site 4
Location	MJ Bananas Davidson Road, Euramo QLD	Cherryvale Plantation Bruce Highway Upper Murray QLD	Lardi Syndicate Road Tully Valley QLD	Brighton East Feluga Road Feluga QLD
Crop	Bananas	Bananas	Bananas	Bananas
Variety	Cavendish	Cavendish	Cavendish	Cavendish
Plot size	4 plants/plot	4 plants/plot	4 plants/plot	4 plants/plot
Spray equipment	Small portable knapsack sprayer	Small portable knapsack sprayer	Small portable knapsack sprayer	Small portable knapsack sprayer
Number of Applications	2 × Butt sprays – spray was applied to the base of the pseudostem and surrounding soil.	2 × Butt sprays – spray was applied to the base of the pseudostem and surrounding soil.	2 × Butt sprays – spray was applied to the base of the pseudostem and surrounding soil.	2 × Butt sprays – spray was applied to the base of the pseudostem and surrounding soil.
Application rate	100 g diazinon/100 L (0.6 g diazinon/plant)	100 g diazinon/100 L (0.6 g diazinon/plant)	100 g diazinon/100 L (0.6 g diazinon/plant)	100 g diazinon/100 L (0.6 g diazinon/plant)
Re-application intervals	14 days	14 days	14 days	14 days
Volume of application	600 mL/plant (3000 L/ha)	600 mL/plant (3000 L/ha)	600 mL/plant (3000 L/ha)	600 mL/plant (3000 L/ha)
Sampling times	0 DALT	0 DALT	0 DALT	0 DALT
Sample collection	24 bananas (~2 kg) were collected, taking 2 fingers from each of the top, middle and lowest hands of 4 bunches.	24 bananas (~2 kg) were collected, taking 2 fingers from each of the top, middle and lowest hands of 4 bunches.	24 bananas (~2 kg) were collected, taking 2 fingers from each of the top, middle and lowest hands of 4 bunches.	24 bananas (~2 kg) were collected, taking 2 fingers from each of the top, middle and lowest hands of 4 bunches.
Sample storage	Frozen within 2 hours of collection	Frozen within 2 hours of collection	Frozen within 2 hours of collection	Frozen within 2 hours of collection
In-field trial dates	1 February 2002 to 15 February 2002	1 February 2002 to 15 February 2002	1 February 2002 to 15 February 2002	1 February 2002 to 15 February 2002
Completion date for analytical phase	31 March 2002	31 March 2002	31 March 2002	31 March 2002
Storage period	<2 months	<2 months	<2 months	<2 months

The levels of diazinon residues in bananas were determined using a GLC method with phosphorus-specific flame photometric detection (GC-PFPD). Briefly, 50 g aliquots of banana were blended with acetone. The extract was then partitioned against hexane/dichloromethane (1:1, v/v). After drying over sodium sulfate, the extract was concentrated using a rotary evaporator. The concentrated extract was reconstituted in acetone before being analysed for its diazinon content using a GC-PFPD method. Validation data demonstrated that the method limit of reporting (LOR) was 0.02 mg/kg, and recoveries from banana samples fortified with 0.021, 0.105 and 0.210 mg diazinon/kg were 119 %, 94.3 % and 114.8 %, respectively.

Results

The levels of diazinon residues in bananas that were treated with two butt spray applications of 0.6 g diazinon/plant at 14 day intervals (ie 1× the maximum proposed rate) are tabulated below. Residue values have not been corrected for method recoveries.

Trial Number	Trial location	Treatment regimen	Sampling time (DALT)	Diazinon residues (mg/kg)
1	Euramo QLD	Untreated control	0	<0.02
		2 applications of 0.6 g ai/plant, applied at 14 day intervals (1×)	0	<0.02
2	Upper Murray QLD	Untreated control	0	<0.02
		2 applications of 0.6 g ai/plant, applied at 14 day intervals (1×)	0	<0.02
3	Tully Valley QLD	Untreated control	0	<0.02
		2 applications of 0.6 g ai/plant, applied at 14 day intervals (1×)	0	<0.02
4	Feluga QLD	Untreated control	0	<0.02
		2 applications of 0.6 g ai/plant, applied at 14 day intervals (1×)	0	<0.02

Comment

When bananas are treated with 2 butt spray applications of 0.6 g diazinon/plant at 14 day intervals (ie 1× the maximum proposed rate), residues in bananas immediately after the second treatment were all below the limit of reporting (ie <0.02 mg/kg). Based on these data, an MRL of *0.02 mg/kg is considered appropriate to cover the occurrence of diazinon residues in treated bananas.

Reference

Trials to determine the level of diazinon in mushrooms at harvest following application to the casing layer. (2004) Report No.: AMGA/01/04. Study Director: A. Clift, University of Sydney, NSW. Author: K. Bodnaruk, West Pymble, NSW. Test Facility: Agrisearch Analytical Pty Limited, Rozelle, NSW.

Experiment

Seven confirmatory Australian residues trials were conducted with diazinon in mushrooms. In each trial, diazinon was applied to the casing layer (ie after mycelial growth, but before the start of fruiting body formation) at a rate of 3.2 g diazinon/m^2 (equivalent to 24 g diazinon/tonne of casing mix). Mushroom caps were harvested after the first flush. Details of each of the trials are tabulated below:

Details	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7
Location	ς MLMU, Sydney Uni	ς MLMU, Sydney Uni	ς MLMU, Sydney Uni	ς MLMU, Sydney Uni	ς MLMU, Sydney Uni	ς MLMU, Sydney Uni	ς MLMU, Sydney Uni
Crop	Mushrooms	Mushrooms	Mushrooms	Mushrooms	Mushrooms	Mushrooms	Mushrooms
Variety	Sylvan A15	Sylvan A15	Sylvan A15	Sylvan A15	Sylvan A15	Sylvan A15	Sylvan A15
Plot size	one bag (22 kg) compost + casing, 0.18 m^2.	one bag (22 kg) compost + casing, 0.18 m^2.	one bag (22 kg) compost + casing, 0.18 m^2.	one bag (22 kg) compost + casing, 0.18 m^2.	one bag (22 kg) compost + casing, 0.18 m^2.	one bag (22 kg) compost + casing, 0.18 m^2.	one bag (22 kg) compost + casing, 0.18 m^2.
Number of applications	one	one	one	one	one	one	one
Timing of application	℘ Diazinon applied to casing material and mixed with a ribbon mixer. Casing applied 10-15 days post spawning.						
Application rate	3.2 g diazinon/m^2	3.2 g diazinon/m^2	3.2 g diazinon/m^2	3.2 g diazinon/m^2	3.2 g diazinon/m^2	3.2 g diazinon/m^2	3.2 g diazinon/m^2
Adjuvant used	None	None	None	None	None	None	None
Volume of application	7.6 mL product (6 g of diazinon) was diluted in 10 L water prior to application	7.6 mL product (6 g of diazinon) was diluted in 10 L water prior to application	7.6 mL product (6 g of diazinon) was diluted in 10 L water prior to application	7.6 mL product (6 g of diazinon) was diluted in 10 L water prior to application	7.6 mL product (6 g of diazinon) was diluted in 10 L water prior to application	7.6 mL product (6 g of diazinon) was diluted in 10 L water prior to application	7.6 mL product (6 g of diazinon) was diluted in 10 L water prior to application
Rainfall in 24 hours after application	Trials conducted in controlled environment rooms (as per commercial practice)						
Sampling times	First flush – at 16 and 19 DAT	First flush – at 19 and 21 DAT	First flush – at 19 DAT	First flush – at 24 and 25 DAT	19, 21, 24 and 38 DAT	24, 27 and 44 DAT	26 and 36 DAT
Sample collection	600 g (~30 fruit) were collected, trimmed and any adhering casing material was brushed off.						
Sample storage	Frozen within 2 hours of collection	Frozen within 2 hours of collection	Frozen within 2 hours of collection	Frozen within 2 hours of collection	Frozen within 2 hours of collection	Frozen within 2 hours of collection	Frozen within 2 hours of collection
'In-field' trial dates	13 Mar 2003 to 14 Apr 2003	6 Aug 2003 to 11 Sept 2003	20 Aug 2003 to 23 Sept 2003	15 Oct 2003 to 18 Nov 2003	28 Oct 2003 to 15 Dec 2003	10 Dec 2003 to 5 Feb 2004	14 Jan 2004 to 4 Mar 2004
Completion date for analytical phase	May 2004	May 2004	May 2004	May 2004	May 2004	May 2004	May 2004
Storage period	~13 months	~9 months	~9 months	~7 months	~6 months	~5 months	~4 months

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℘ Cultivated mushrooms are usually grown in climate-controlled rooms. The fungal inoculum or "spawn" is added to a pasteurised substrate in growing containers or beds. After the fungal strands (mycelia) have spread throughout the compost, a layer of peat or soil (the "casing") is added. The casing protects the compost from drying out, and allows for formation of the fruiting bodies (mushrooms). The mushrooms begin appearing about 6 weeks after spawning, and continue appearing in flushes about 7-10 days apart for a further 6-8 weeks. The first three flushes are the most productive. The cap and a small section of connected stem are usually harvested before the caps are fully expanded.

DAT: Days After Treatment

Analytical method

The levels of diazinon residues in mushrooms were determined using a GC/MS method. Briefly, 50 g samples of mushrooms were blended with acetone. After filtration, the extract volume was made up to 250 mL with acetone. A 50 mL aliquot of the extract was saturated with sodium chloride, before being partitioned against dichloromethane. The organic phase was concentrated using a rotary evaporator, and then cleaned up by gel permeation chromatography. The eluate containing diazinon was concentrated by rotary evaporation, and then made to 5 mL with toluene. The diazinon content of samples was determined using a GC/MS method [m/e 304 amu (target diazinon), m/e 137, 179 and 152 amu (qualifying)], and an external standard calibration curve. Validation data demonstrated that the limit of quantitation (LOQ) of the method was 0.05 mg/kg, the limit of detection (LOD) was 0.01 mg/kg, and recoveries from mushroom samples fortified with 0.064 mg diazinon/kg ranged from 87 to 90 % (mean ± SD = 88 ± 1.2 %, n=3). The detector response was also demonstrated to be linear (r^2 = 1.000) over the concentration range 0.064 to 0.322 mg/kg.

Results

The levels of diazinon residues in mushrooms grown in casing that had been treated with a single application of 3.2 g diazinon/m^2 (equivalent to 24 g diazinon/tonne of casing mix) are tabulated below. Residues have not been corrected for method recoveries (LOD = 0.01 mg/kg; LOQ = 0.05 mg/kg).

Trial Number	Trial location	Treatment Regimen	Sampling time (DAT)	Diazinon residues (mg/kg)
1	[ζ]MLMU, Sydney Uni	Untreated control	--	<0.01
		Mushroom casing treated with 3.2 g diazinon/m^2.	16	<0.01
			19	<0.05
2	[ζ]MLMU, Sydney Uni	Untreated control	--	<0.01
		Mushroom casing treated with 3.2 g diazinon/m^2.	19	<0.05
			21	<0.01
3	[ζ]MLMU, Sydney Uni	Untreated control		<0.01
		Mushroom casing treated with 3.2 g diazinon/m^2.	19	<0.05
4	[ζ]MLMU, Sydney Uni	Untreated control	--	<0.01
		Mushroom casing treated with 3.2 g diazinon/m^2.	24	(sample missing)
			25	<0.01
5	[ζ]MLMU, Sydney Uni	Untreated control	--	<0.01
		Mushroom casing treated with 3.2 g diazinon/m^2.	19	<0.01
			21	<0.01
			24	<0.01
			38	<0.01
6	[ζ]MLMU, Sydney Uni	Untreated control	--	<0.01
		Mushroom casing treated with 3.2 g diazinon/m^2.	24	<0.01
			27	<0.01
			44	<0.01
7	[ζ]MLMU, Sydney Uni	Untreated control	--	<0.01
		Mushroom casing treated with 3.2 g diazinon/m^2.	26	<0.01
			36	<0.01

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Comment

When mushrooms are grown in casing that has been treated with 3.2 g diazinon/m^2 (equivalent to 24 g diazinon/tonne of casing mix; 1× maximum registered rate), residues in the first flush of mushrooms (~3 weeks post-treatment) are all below the Limit of Quantitation of the method (<0.05 mg/kg). Based on these data, an MRL of *0.05 mg/kg is considered appropriate to cover the occurrence of diazinon residues in mushrooms grown in treated casing.

Reference

Trials to determine the level of diazinon in onions at harvest following two applications as a butt spray (sic). (2002) Report No. VX01029. Protocol No. ECR-002. Study Director: K. Bodnaruk, West Pymble NSW. Author: K. Bodnaruk, West Pymble NSW. Analytical Laboratory: Victorian State Chemistry Laboratory, Werribee, VIC.

Experiment

Three confirmatory Australian residues trials were conducted with diazinon in onions. Details of each of the trials are tabulated below:

Details	Site 1	Site 2	Site 3
Location	"Belvedere" Sturt Highway Narranderra NSW 2700	Carosa Road, Wanneroo, WA 6065	Forthside Vegetable Res Station Forth, TAS 7310
Crop	Onions (bulb)	Onions (bulb)	Onions (bulb)
Variety	Creamgold	Early flat	Breeding line
Plot size	10 × 10 m	40 × 1.3 m	4 × 20 m
Spray equipment	Small portable knapsack sprayer	Compressed air hand held boom sprayer	Gas powered constant pressure mini boom
Number of Applications	3 × foliar applications	3 × foliar applications	3 × foliar applications
Application rate	560 g diazinon/ha	560 g diazinon/ha	560 g diazinon/ha
Re-application intervals	11, 9 days	10, 10 days	10, 11 days
Volume of application	200 L/ha	350 L/ha	253 L/ha
Sampling times	3 and 14 DALT	0 and 15 DALT	0 and 14 DALT
Sample collection	12-15 bulbs collected (>3 kg), excess dirt shaken off	12 bulbs collected (~2 kg), stems leaves and roots removed and bulbs brushed to remove sand.	12 onions collected, roots and tops were removed.
Sample storage	Frozen within 2 hours of collection	Frozen within 1 hour of collection.	Frozen within 1 hour of collection.
In-field trial dates	26 January 2002 to 1 March 2002	29 August 2002 to 2 October 2002	21 January 2002 to 25 February 2002
Completion date for analytical phase	16 August 2002	23 December 2002	16 August 2002
Storage period	~6 months	~3 months	~6 months

The levels of diazinon residues in onions were determined using a GLC method with pulsed flame photometric detection (GC-PFPD). Briefly, 50 g aliquots of homogenised onion were extracted with acetone. The extract was then partitioned against dichloromethane. After drying over sodium sulfate, the extract was concentrated in a boiling water bath, with inversion into hexane. Finally the extract was analysed by GC-PFPD, and quantified using an external standard calibration curve. Validation data demonstrated that the method limit of reporting (LOR) was 0.01 mg/kg, and recoveries from onion samples fortified with 0.30 and 0.71 mg diazinon/kg were 78 % and 99 %, respectively.

Results

The levels of diazinon residues in bulb onions that were treated with three foliar applications of 560 g diazinon/ha at 10 day intervals (ie 1× the maximum proposed rate) are tabulated below. Residue values have not been corrected for method recoveries.

Trial Number	Trial location	Treatment regimen	Sampling time (DALT)	Diazinon residues (mg/kg)
1	Narranderra NSW	Untreated control	3	0.057
		3 applications of 560 g ai/ha, applied at 10 day intervals (1×)	3	0.073
			14	0.036
2	Wanneroo, WA	Untreated control	0	<0.01
		3 applications of 560 g ai/ha, applied at 10 day intervals (1×)	0	<0.01
			14	<0.01
3	Forth, TAS	Untreated control	0	0.032
		3 applications of 560 g ai/ha, applied at 10 day intervals (1×)	0	0.032
			14	0.024

Comments

- There are some apparent anomalies in the residue results from the three Australian confirmatory trials conducted with diazinon in onions. In two of the trials (NSW and Tasmania), detectable levels of diazinon were reported for the untreated control samples. It is unclear whether there has been an error in the collection procedure for control samples ie whether samples were collected from the untreated plots, or whether the control samples were collected from the correct plots, but were subsequently contaminated with diazinon. It is considered unlikely that the analytical laboratory was the source of the error, since the results from the WA trial are not anomalous.
- Since the source of error in the results for the untreated control samples is unclear, it is not considered appropriate to correct the residue results for the treated samples (ie by subtracting the control levels from the treated residue concentrations).
- When bulb onions are treated with 3 foliar applications of 560 g diazinon/ha at 10 day intervals (ie at the maximum 1× registered use rate), residues in onions at 14 DALT ranged from <0.01 mg/kg to 0.036 mg/kg. Based on these data, an MRL of 0.05 mg/kg is considered appropriate to cover the occurrence of diazinon residues in treated onions.

Reference

Submission of residue field trial data for determination of a NRA MRL in pineapples for diazinon. (2003) Sponsor: A&C Rural, Narangba, QLD. Author: D. Corbett, A&C Rural Pty Limited. Analytical Laboratory: Agrisearch Analytical Pty Limited, Rozelle, NSW.

Experiment

Three confirmatory Australian residues trials were conducted with diazinon in pineapples. Details of each of the trials are tabulated below:

Details	Site 1	Site 2	Site 3
Location	Palmwoods, SE QLD	Yeppoon, Central QLD	Glasshouse Mountains, QLD
Crop	Pineapples	Pineapples	Pineapples
Variety	Smooth Cayenne	Smooth Cayenne	Smooth Cayenne
Plot size	37 m^2 (2 commercial rows)	20 m^2	20 m^2
Spray equipment	Portable CO_2 pressurised sprayer	Portable CO_2 pressurised sprayer	Portable CO_2 pressurised sprayer
Number of Applications	26	2	2
Application rate	2.4 kg diazinon/ha (spray entire plant to run-off)	2.4 kg diazinon/ha (spray entire plant to run-off)	2.4 kg diazinon/ha (spray entire plant to run-off)
Re-application intervals	14 days	7 days	7 days
Volume of application	3000 L/ha	3000 L/ha	3000 L/ha
Sampling times	3, 7, 14 and 21 DALT	7 and 14 DALT	7 and 14 DALT
Sample collection	12 fruit with crowns removed (~15-20 kg). Fruit were crushed and homogenised, then 3 × 500 g aliquots were frozen.	12 fruit with crowns removed (~17-24 kg). Fruit were crushed and homogenised, then 3 × 500 g aliquots were frozen.	12 fruit with crowns removed (~18-19 kg). Fruit were crushed and homogenised, then 3 × 500 g aliquots were frozen.
Sample storage	Frozen at –20 °C within 24 h of collection.	Frozen at –20 °C within 24 h of collection.	Frozen at –20 °C within 24 h of collection.
In-field trial dates	2 March 2001 to 26 February 2002	16 January 2002 to 6 February 2002	4 February 2002 to 25 February 2002
Completion date for analytical phase	5 January 2003	5 January 2003	5 January 2003
Storage period	~12 months	~12 months	~11 months

The levels of diazinon residues in pineapples were determined using a GC/MS method. Briefly, 50 g aliquots of homogenised pineapple were blended with acetone. After filtration, the extract was partitioned against dichloromethane. The organic phase was concentrated using a rotary evaporator, then cleaned up on a silica gel column. The eluate containing diazinon was concentrated before being made to volume with toluene. The diazinon content of samples was determined using a GC/MS method (m/e 304 amu (target diazinon), m/e 199 amu and m/e 179 amu (qualifying)). Validation data demonstrated that the method limit of quantitation (LOQ) was 0.01 mg/kg, and recoveries from pineapple samples fortified with 0.0098 mg diazinon/kg ranged from 58 to 100 % (mean ± SD = 74 ± 16 %). The detector response was also demonstrated to be linear (r^2 = 1.000) over the concentration range 0.049 to 0.986 mg/kg.

Results

The levels of diazinon residues in pineapples that were treated with foliar applications of diazinon at a rate of 2.4 kg diazinon/ha are tabulated below. Residue values have not been corrected for method recoveries. (LOD = 0.005 mg/kg; LOQ = 0.01 mg/kg)

Trial Number	Trial location	Treatment regimen	Sampling time (DALT)	Diazinon residues (mg/kg)
1	Palmwoods, SE QLD	Untreated control	7/14†	<0.005
		26 applications of 2.4kg ai/ha, applied at 14 day intervals (1×)	3	0.07
			7	0.03
			14	0.04
			21	<0.01
2	Yeppoon, Central QLD	Untreated control	7/14†	<0.005
		2 applications of 2.4 kg ai/ha, applied at 7 day interval (1×)	7	0.01
			14	<0.005
3		Untreated control	7/14†	<0.005

Trial Number	Trial location	Treatment regimen	Sampling time (DALT)	Diazinon residues (mg/kg)
	Glasshouse Mountains, QLD	2 applications of 2.4 kg ai/ha, applied at 7 day interval (1×)	7	0.03
			14	<0.01

†Untreated control samples were collected at 7 and 14 DALT. However, details of which of the control samples were analysed were not provided.

Comment

When pineapples were treated with foliar applications of diazinon at a rate of 2.4 kg diazinon/ha at 7-14 day intervals (ie 1× the maximum registered rate), residues in pineapples at 14 DALT (ie the proposed harvest WHP) were <0.005 mg/kg, <0.01 mg/kg and 0.04 mg/kg. Based on these data, an MRL of 0.05 mg/kg is considered appropriate to cover the occurrence of diazinon residues in treated pineapples.

3.7 Appendix 2: Dietary Intake Calculations

NEDI: based on the mean consumption of the relevant Australian consumers aged 2 years and above

Intake Calculation for **DIAZINON**

(ADI for Diazinon = 0.001

Commodity	Food Consumption g/kg bw/day		MRL mg/kg	Processing Factor	Estimated Intake mg/kg bw/day	% of ADI
FI 0328 Banana, dwarf	0.0001	*	0.02	1.00	0.000000002	0.0002
FI 0327 Banana	0.3515	*	0.02	1.00	0.00000703	0.703
MO 0105 Edible offal (mammalian)	0.0136		0.03	1.00	0.000000408	0.0408
^MM 0095 Meat (mammalian) [in the fat]	0.1757		0.70	0.70	0.000086093	8.6093
ML 0106 Milks	8.9933		0.02	1.00	0.000179866	17.9866
VO 0450 Mushroom	0.049	*	0.05	1.00	0.00000245	0.245
VA 0385 Onion	0.2767		0.05	1.00	0.000013835	1.3835
HH0740 Parsley	0.0045	T	0.70	1.00	0.00000315	0.315
FI 0353 Pineapple	0.2172		0.05	1.00	0.00001086	1.086
VA 0388 Shallot	0.0061	T	0.50	1.00	0.00000305	0.305
VA 0389 Spring onion	0.0087	T	0.50	1.00	0.00000435	0.435
Total					0.000311094	31.1094

* At or about the limit of determination

**** Equivalent to 31.1**

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ADI - Acceptable Daily Intake

^Consumption figure = MF + 10 % MM

Diazinon Acute Dietary Intake _ 2-6 years Date 2/05/2006

Acute RfD	0.01 mg/kg bw

Commodity

Code	Name	MRL mg/kg	STMR or STMR-P, mg/kg	Process factor	HR or HR-P, mg/kg	Large portion, g/kg bw	Body weight, kg	Large portion, kg	Unit weight, g	% edible portion	Unit weight, edible portion, kg	Variability factor	Case	NESTI, mg/kg bw/day	% acute RfD
Fat Soluble Componds:															
MM 0095	Meat (mammalian)	0.7				13.715	19	0.261			0.000		Case 1	0.0017	17.007
	Muscle 80% consumption				0.02	10.972	19	0.208					-	0.0002	
	Fat 20% consumption				0.54	2.743	19	0.052					-	0.0015	
MO 0105	Edible offal (mammalian)	0.03			0.02	0.847	19	0.016			0.000		Case 1	0.0000	0.169
ML 0106	Milks	0.02	0.02		0.02	76.325	19	1.450			0.000		Case 3	0.0015	15.265
FI 0327	Banana	0.02			0.02	14.147	19	0.269	132	65	0.086	3	Case 2a	0.0005	4.636
VO 0450	Mushrooms	0.05			0.05	2.784	19	0.053			0.000		Case 1	0.0001	1.392
VA 0385	Onion	0.05			0.036	3.356	19	0.064	110	88	0.097	3	Case 2b	0.0004	3.624
^HH 0740	Parsley	0.7			0.7	0.302	19	0.006			0.000		Case 1	0.0002	2.114
FI 0353	Pineapple	0.05			0.04	35.638	19	0.677	1800	67	1.206	3	Case 2b	0.0043	42.766
^VA 0388	Shallot	0.5			0.5	0.951	19	0.018	29	72	0.021		Case 1	0.0005	4.755
^VA 0389	Spring onion	0.5			0.5	1.519	19	0.029	17	78	0.013		Case 1	0.0008	7.595

Case 1. Composite sampling data reflect the residue level in the food, based upon HR or HR-P
Case 2. Composite residue data do not reflect the residue level in individual food commodity units.
Case 2a. Unit weight edible portion is less than large portion weight.
Case 2b. Unit weight edible portion exceeds large portion weight.
Case 3. Processed commodity, where bulking or blending means that the STMR-P represents the likely highest residue

DIAZINON Acute Dietary Intake _ 2 years + Date 14/10/2004

Acute RfD	0.01 mg/kg bw

Commodity

Code	Name	MRL mg/kg	STMR or STMR-P, mg/kg	Process factor	HR or HR-P, mg/kg	Large portion, g/kg bw	Body weight, kg	Large portion, kg	Unit weight, g	% edible portion	Unit weight, edible portion, kg	Variability factor	Case	NESTI, mg/kg bw/day	% acute RfD
MM 0095	Meat (mammalian)	0.7				7.791	67	0.522			0.000		Case 1	0.0008	7.978
	Muscle 80% consumption				0.02	6.2328	67	0.418					-	0.0001	
	Fat 20% consumption				0.54	1.24656	67	0.084					-	0.0007	
MO 0105	Edible offal (mammalian)	0.03			0.02	2.927	67	0.196			0.000		Case 1	0.0001	0.585
ML 0106	Milks	0.02	0.02		0.02	29.654	67	1.987			0.000		Case 3	0.0006	5.931
FI 0327	Banana	0.02			0.02	3.821	67	0.256	132	65	0.086	3	Case 2a	0.0001	1.276
VO 0450	Mushrooms	0.05			0.05	1.66	67	0.111			0.000		Case 1	0.0001	0.830
VA 0385	Onion	0.05			0.036	1.838	67	0.123	110	88	0.097	3	Case 2a	0.0002	1.702
^HH 0740	Parsley	0.7			0.7	0.157	67	0.011			0.000		Case 1	0.0001	1.099
FI 0353	Pineapple	0.05			0.04	11.765	67	0.788	1800	67	1.206	3	Case 2b	0.0014	14.118
^VA 0388	Shallot	0.5			0.5	0.458	67	0.031	29	72	0.021		Case 1	0.0002	2.290
^VA 0389	Spring onion	0.5			0.5	0.896	67	0.060	17	78	0.013		Case 1	0.0004	4.480