Exposure to pesticides of fruit growers and effects on reproduction: an epidemiological approach

Johan de Cock

Stellingen

1. Men kan betwijfelen of grenswaarden alleen voor de luchtwegblootstelling, beroepsmatig blootgestelden in de landbouw afdoende beschermen.
(Dit proefschrift)

2. Een grenswaarde voor de beroepsmatige luchtwegblootstelling is niet het juiste uitgangspunt om een huidindicatie vast te stellen.
(Dit proefschrift)

3. De huidblootstelling kan beter worden gerelateerd aan biologische monitoring op grond van studies gericht op specifieke delen van de huid, dan op schattingen van de totale dosis.
(Dit proefschrift)

4. Er zijn aanwijzingen dat de blootstelling aan bestrijdingsmiddelen in de fruitteelt een nadelig effect heeft op de fecundabiliteit.
(Dit proefschrift)

5. Gezien het traditionele familie karakter van veel agrarische bedrijven, waarbij vaak de arbeids- en woonomgeving nauw aan elkaar zijn verbonden, is de populatie 'at risk' groter dan alleen de populatie werkenden.

6. De moeite die een fruitteler moet doen om aan de eisen van de consument te voldoen, staat in geen verhouding tot de wijze waarop de consument bij de aankoop vaak met fruit omgaat.

7. Bij werken met bestrijdingsmiddelen betekent schoonmaken zo veel als vies worden.

8. Dat muziek geen lawaai is, wil nog niet zeggen dat lawaaidoofheid in de muziek niet voorkomt.

9. Het is verbazingwekkend dat mensen 's ochtends vroeg op een vol perron van een metrostation in London stiller zijn dan in een concertzaal tussen twee delen van een concert.

10. De term 'black box' in de epidemiologie is vergelijkbaar met de term idiopatisch in de geneeskunst.

11. Een waarschuwing over de gezondheid zoals vermeld op een pakje sigaretten zou ook op een auto niet misstaan.

12. De risico's van een bestrijdingsmiddel worden niet kleiner door het een gewasbeschermingsmiddel te noemen.

Stellingen, behorende bij het proefschrift 'Exposure to pesticides of fruit growers and effects on reproduction: an epidemiological approach' van Johan Sytse de Cock, Wageningen, 10 november 1995

Exposure to pesticides of fruit growers and effects on reproduction: an epidemiological approach

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Exposure to pesticides of fruit growers and effects on reproduction: an epidemiological approach

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ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen,
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A steering committee for this study was founded in 1989 consisting of representatives of fruit growers, the Dutch National Fruit Growers' Organization (NFO), the Board of Agriculture (Landbouwschap), The Joint Services for Occupational Safety and Health (STIGAS), the Information and Knowledge Centre (IKC), the Institute of Agricultural and Environmental Engineering (IMAG), and the Product Board for Vegetables and Fruit (PGF).

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Contents

ABSTRACT
Exposure to pesticides of fruit growers and effects on reproduction:
an epidemiological approach

PhD Thesis, Department of Epidemiology and Public Health, Department of Air Quality, Wageningen Agricultural University, P.O. Box 238, 6700 AE Wageningen, The Netherlands, November, 1995

Johan Sytse de Cock

In this thesis the exposure to pesticides of fruit growers in The Netherlands was studied as well as its relation to reproductive health effects. The most commonly used fungicide, *captan*, was used as a marker for exposure. Several exposure studies were carried out during application of captan, and work in the orchards. As exposure per unit of time was in the same order of magnitude for very different tasks such as application and re-entry, individual time spent on different tasks is crucial for estimating total exposure. As day-to-day variability in exposure was high, within as well as between workers, repeated measurements are necessary to estimate individual exposure accurately. For the application, respiratory exposure was predominantly related to the preparation of tank mixtures. Cabin use on the tractor was the most prominent determinant of dermal exposure during spraying. During re-entry dislodgeable foliar residue was the most prominent determinant of exposure for both respiratory and dermal exposure. Uptake of captan into the human body was measured with biological monitoring of tetrahydrophtalimide in urine of exposed farmers after application. Urine levels were related to dermal exposure of specific skin areas. The consequences of these findings for the way a 'skin notation' is set, is discussed. Another study was carried out to evaluate methods for subjective assessment of pesticide exposure in fruit growing by experts. Experts seem to recognize the most important determinants of exposure. In an epidemiological study time to pregnancy was used to study effects of pesticide exposure. In addition, the relationship with offspring sex ratio was explored. The findings of the study on time to pregnancy indicate that an adverse effect of exposure to pesticides on fecundability is likely. As very few epidemiological studies have been carried out on the effects of pesticides on reproduction, no firm conclusions can be drawn about specific pesticides or other related factors responsible for the observed effect on time to pregnancy or on offspring sex ratio. The indication, however, justifies more attention for possible effects of pesticide exposure on reproduction. In this light, reduction of exposure to pesticides as far as achievable is needed. The validity of the observed effects on reproduction and the underlying mechanisms have to be elucidated.

Keywords: pesticides, captan, THPI, fruit growers, occupational exposures, biological monitoring, time to pregnancy, sex ratio.

CHAPTER 1

Introduction

An increase in the number of pesticides[1] and in total amounts used during the last decades have led to growing attention to possible adverse effects on human health. The population occupationally exposed consists of workers in agriculture, in public parks and gardens, and pesticide production workers.

In the early years of pesticide use, research on adverse health effects most often concerned studies on acute effects and fatal intoxications. The effects were mainly a result of poisoning or effects after high accidental exposures and concerned a single case or a few cases at a time. Knowledge on adverse effects was mainly based on toxicological data from animal studies and human case reports.

More recent epidemiological studies, often are carried out on a larger scale and cover a diversity of health endpoints such as neurotoxic, immunotoxic, carcinogenic, reprotoxic, and developmental effects. Mostly, chronic effects of long term exposure are the focus in these studies.

Changes in pesticide use over the years are due to developments in the production technology of new pesticides, changes in culture techniques, and regulations of

[1] Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), a pesticide is defined as "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating, any insects, rodents, nematodes, fungi, or weeds, or any other forms of life declared to be pests, and any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant" (NRC, 1993).

The Dutch pesticide act defines a pesticide as an agent or mixture of agents, including micro organisms and viruses, meant to kill animal species and plants, which damage agricultural and horticultural crops. This definition includes herbicides, weed killers, growth regulators and agents to kill parasites on animals or in animal houses (Plantenziektekundige Dienst, 1993; Bestrijdingsmiddelenwet, 1995).

governments based on mainly toxicological research. Furthermore, attention of the media to developments in pesticide use and research on health effects of pesticides on humans may have played an important role on the public opinion and awareness as well.

Public concern in the United States about exposure to residues in food and water led to a workshop on potential chronic health effects of pesticides (Baker *et al.* 1990). The main conclusion was that chronic health effects in humans are most likely to result from excessive pesticide exposure that might occur in occupational settings. The risks for the general population from food residues are generally expected to be low. On the other hand, it was concluded that there is a need to improve the methods for estimating exposure to pesticide residues in food and for setting tolerances to safeguard the health of infants and children (NRC, 1993).

The fact that very few epidemiological studies have been conducted in the area of pesticide exposure was partly attributed to difficulties in identifying and quantifying exposures to different pesticides over time and the existence of many potential confounding factors (Baker *et al.* 1990). This is not surprising when one realises that the Environmental Protection Agency (EPA) in the U.S. estimates that there are about 600 active ingredients in 45,000 - 50,000 pesticide formulations (Baker, 1990). In The Netherlands the number of registered active ingredients is around 500 of which 300 for agricultural purposes (CBS, 1990). Epidemiological studies are, in addition to the large number of different pesticides, further complicated by changes over time. In The Netherlands in the period of 1965-1988, for example, 248 new active ingredients were introduced, while 162 others disappeared from the market (CBS, 1990).

In many countries regulations concerning the registration of pesticides have become more strict during recent years. The policy is to reduce the total amount being used by the introduction of alternative pest control technologies, such as integrated pest management. In The Netherlands a 'Multi-Year Crop Protection Plan' came into force, aimed at reducing the volume used in 1984-1988 by 50% by the year 2000 to reduce the immision of pesticides to the environment and decrease the dependency on chemical crop protection.

In The Netherlands limit values exist for food, (the Acceptable Daily Intake, ADI), for ground water, for lakes and streams, and for soil. For occupational exposure the Maximum Accepted Concentration (MAC) exists, which aims to prevent health impairment of workers and also their progeny (Boleij *et al.*, 1995).

Epidemiological research to evaluate occupational exposure to pesticides of a population of farmers is complex, since it typically concerns exposure to a mixture of agents. Furthermore, contribution to uptake through the different exposure routes (skin, respiratory and gastrointestinal tract) not only depends on physicochemical properties of the pesticides, but also on personal factors, and occupational and environmental conditions.

It has long been recognized that occupational exposure of the skin, may lead to dermal uptake. A recent review of exposure assessment guidelines for pesticides concluded, however, that still little progress in the area of methodologies for dermal exposure monitoring has been made (Curry and Iyengar, 1992; Dost, 1994).

Factors which determine percutaneous absorption of chemicals include exposure factors, as concentration and type of vehicle, extent of skin contact (*i.e.* the area covered), duration, and efficiency of removal (Grandjean, 1990). Other factors involve the skin, such as skin permeation, which depends on the skin region, skin disease and wounds, and skin hydratation in combination with recent contact with damaging chemicals. Furthermore, occlusion and temperature are important.

Surprisingly, occupational exposure limits (OEL) are lacking for several pesticides and mostly only consider respiratory exposure. For the pesticide top-10 in fruit growing only for four an occupational exposure limit has been established (Table 1).

Data on occupational exposures to pesticides have been collected over many years. Studies tend to consist of small data sets, while large differences in exposure circumstances occur. Repeated exposure measurements of the same workers in order to deal with the usually high day-to-day variability are scarce. Furthermore, exposure data are often difficult to compare since different methods for exposure measurement have been used. Part of the data concern unpublished data submitted in the framework of registration procedures. Recently, exposure data and published proposals for generic data bases were reviewed (van Hemmen, 1993). It was concluded that these data bases can be used as a first step for risk assessment. Surprisingly, generic data bases in the literature were presented for exposure during mixing/loading and application but not for re-entry, *i.e.* exposure during tasks in the orchards other than the application.

Fruit growing

As no information on occupational exposure of fruit growers in The Netherlands was available, in 1989 a preliminary study among fruit growers applying pesticides was carried out (Amelsvoort *et al.*, 1989). The main reasons to study fruit growers in our study were that: 1) fruit growers tend to use a large number of different pesticides; 2) fruit growers use some of these pesticides frequently and in considerable amounts; 3) data on exposure were not available for this population and; 4) exposure to pesticides mostly concerns outdoor exposure.

Fruit growing in The Netherlands traditionally is a family business, and occupational exposure is not restricted only to the fruit farmer. In 1992, the population working on around 3,000 farms, consisted of a workforce of 4659 males and 2064 females (LEI, 1992). The male farmer sometimes assisted by one of his sons, will almost without exception perform the applications. The whole family, however, tend to participate in farm work and may be occupationally exposed during tasks in the orchards. Furthermore, since the farm generally is located between the orchards, residential exposure to residues of pesticides of family members may occur. Another group of potentially exposed are the seasonal workers, who are hired mainly to assist during harvesting.

Marker for exposure

As it is hardly possible to measure exposure to each pesticide used in fruit growing separately, the most frequently used fungicide, *captan*, was chosen as a marker for exposure. For consumption crops, apples showed the highest use of pesticides in general in The Netherlands (31 kg active ingredient per hectare). *Captan* is taking with 0.4×10^6 kg/year, the third place of the most frequently used active ingredients and almost 90% was used on apples and pears. An amount of 19.4 kg/ha was sprayed on apples, which was with 16.5 sprayings (1992) around 1.15 kg/ha per spraying. (CBS, 1994).

Captan, a phtalimide fungicide, can cause allergic contact dermatitis and is a suspected carcinogen (Grandjean, 1990). The percutaneous absorption in rats after 72h is about 4% at high doses, but about 10-fold higher at very low dose levels (Shah *et al.*, 1987; Grandjean 1990). In the 'Captan health and safety guide' (WHO, 1990) the carcinogenic potential of *captan* is considered low. It was, nevertheless, recommended that this pesticide should be applied with caution, particularly where residues in food can result. The maximum residue limit (MRL) on pip fruit is 3 mg/kg (Bestrijdingsmiddelenwet, 1995). The recommended TLV by the US ACGIH (1992) is 5 mg/m^3 in air. A Joint FAO/WHO Meeting on Pesticide Residues has established an ADI of 0-0.1 mg/kg body weight (WHO 1990).

Several exposure studies on *captan* in outdoor fruit growing have been carried out (Oudbier, 1974; Hansen, 1978; McJilton, 1983; Zweig *et al.*, 1983, 1985; Winterlin *et al.*, 1984, 1986; Ritcey *et al.*, 1987; Stamper *et al.*, 1987; Fenske 1989). Due to differences in measured tasks, and crop type (strawberries, apples, pears, grape), and differences in measurement- and analytical methods and climatic exposure conditions, it is difficult to compare these studies directly. Respiratory exposure varied in these studies from 0.86-197 μg hr^{-1}, assuming a ventilation rate of 20 L/min. Dermal exposure[2] varied roughly from 3-110 mg hr^{-1} for application of *captan* and 2-50 mg hr^{-1} for re-entry tasks.

Captan has a MAC of 5 mg/m^3 for respiratory exposure and no 'skin notation'[3] (ISZW, 1995), and is put on the agenda of the Dutch Expert Committee on Occupational Standards (DECOS) for re-evaluation (ISZW, 1995).

Exposure measurement techniques

Different techniques for dermal exposure measurements have been described (Durham *et al.*, 1962; Chester, 1993; WHO, 1982). The patch and the rinse techniques are most often used. For respiratory exposure most often personal air samplers are used to measure inhalable dust or exposure to gases and vapours (Boleij *et al.*, 1995).

Biological monitoring, to quantify a metabolite in urine or blood of exposed workers has the advantage that it actually measures uptake into the body via different exposure routes. Major disadvantages are that it is often expensive and time consuming or

[2] Mostly estimates of total dermal exposure

[3] According to The Dutch Expert Committee on Occupational Standards (DECOS), a skin notation should be applied when the amount absorbed by both arms and forearms in one hour could amount to more than 10% of the amount absorbed via the lungs on exposure to the Occupational Exposure Limit (OEL) for eight hours (ECETOC, 1993)

a suitable method is not available. For *captan*, a method has been proposed to measure tetrahydrophtalimide, a metabolite of *captan*, in urine (van Welie *et al.*, 1991).

Epidemiological studies often involve effects of long term exposures, which occur long before the manifestation of disease. This may result in problems because of the absence of adequate monitoring in the past (Goldberg, 1993). Expert assessments to subjectively characterize occupational exposure have been used mainly in industry (Kromhout *et al.*, 1987; Hertzman *et al.*, 1988; Hawkins *et al.*, 1989; Teschke *et al.*, 1989; Post *et al.*, 1991). To see whether experts can estimate a complex situation of outdoor exposure in agriculture, studies have been carried out as part of the EC concerted action "Retrospective evaluation of occupational exposures in cancer epidemiology" (Hémon *et al.*, 1993).

Effects on reproduction

At the time of the first exposure studies among fruit growers, physicians of the Academic University Hospital of Utrecht in The Netherlands raised a question about possible reproductive effects of exposure to pesticides. They suspected a link between pesticide use and infertility among men who visited the fertility unit of the clinic. Since exposure data for fruit growers were available and fruit growers are potentially exposed to many different chemicals, an explorative study on reproductive effects among this population was set up.

Initially, time to pregnancy, suggested by Baird *et al.* (1986) was used as outcome variable. It is considered a simple measure useful in epidemiological studies to determine effects of environmental exposures on fecundability. The fecundability of a couple is the probability of conception for each menstrual cycle and is the inverse of time to pregnancy. This measure was chosen because data can be obtained easily by a face-to-face interview. Fecundability, which depends on many different biological processes in the female as well as the male, seemed a good measure here, because no specific hypothesis could be formulated about the possible underlying mechanisms. Therefore, more invasive methods to measure reproductive effects were considered to be less informative.

In a later stage, in reply to a letter to the editor on the time to pregnancy study (James, 1995), also offspring sex ratio was studied in this population. Some weak scientific evidence states that exposure to pesticides might affect offspring sex ratio. In an earlier study in 1987 an excess of daughters among the offspring of a small group of male 1,2-dibromo-3 chloropropane (DBCP) applicators was observed (Potashnik *et al.*, 1987).

Objectives

The objectives in this study were to characterize occupational exposure in fruit growing in The Netherlands to find determinants of exposure in order to be able to design hazard control strategies and to evaluate if current exposure to pesticides might have adverse effects on reproduction.

Although, findings from this study can form a basis to design hazard control strategies in fruit growing, measures to reduce exposure to pesticides in fruit growing will not be discussed in detail in this thesis.

Outline of the study

In 1989 a postal questionnaire was anonymously sent to all 2,730 members of the Dutch National Fruit Growers' Organization (NFO). Basic information was gathered on orchard type, population, equipment used, performed tasks, used pesticides, duration of treatments and frequency of pesticide use. Among the responding population (41%) several studies were carried out as outlined in figure 1.

CONTENTS

Based on exposure measurements of several field surveys among fruit growers during different tasks performed on the farms, Chapter 2 describes the variability of dermal and respiratory exposure and potential exposure in the home environment. Determinants of exposure from statistical linear modelling are discussed in Chapter 3. As for large scale epidemiological research it is often impossible to carry out large scale exposure measurements, a study was carried out to evaluate methods for subjective assessment of pesticide exposure in fruit growing by experts. This study, described in Chapter 4, was part of the EC concerted action 'Retrospective evaluation of occupational exposures in cancer epidemiology' (Hémon and Goldberg, 1994). In Chapter 5 the relevance of measured external exposure is discussed in relation to actual uptake of pesticides into the human body. Its significance for the choice of dermal exposure measures for epidemiological study purposes is addressed and also the consequences for assigning a 'skin notation'. Characteristics determining exposure from questionnaires, supported by actual exposure measurements were used in an explorative study on the relation between fecundability and occupational exposure to pesticides in fruit growing. This study, is described in Chapter 6. Additionally, offspring sex ratio as an indicator of reproductive hazards associated with pesticides was explored. This study was carried out among the same population as the study on time to pregnancy, which was described in Chapter 7. In Chapter 8 it is discussed if the study population is representative for fruit growers in The Netherlands and if *captan* is a good marker for exposure to pesticides. The findings of the different studies are discussed and finally general conclusions and recommendations are given.

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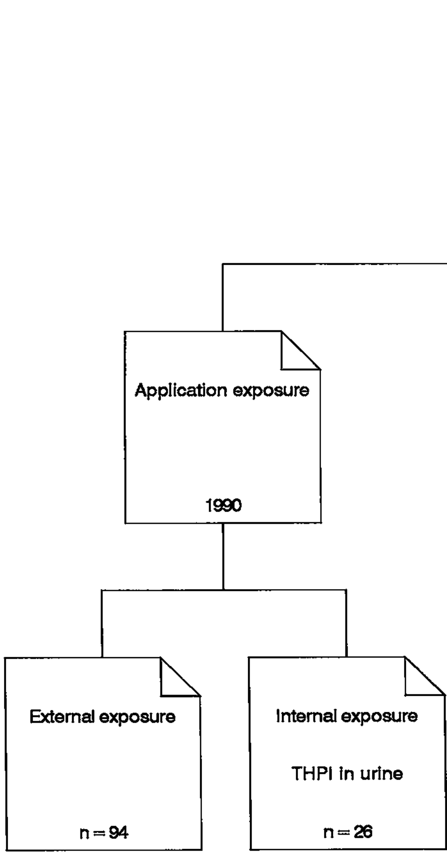
Table 1 Pesticide top-10 in fruit growing in 1989, according to percentage of farms (N = 915), average number of sprayings, and exposure limit values, The Netherlands

Pesticide	Farms(%)	Number of sprayings	TLV[A]	MAC[B]	ADI[C]	LD_{50}[D]	CAS[E]
Fungicides							
Captan	94	9.6	5 (1986)	5	0.013[H]	> 4500[I]	[133-06-2]
Bitertanol	66	3.1	-	-	0.005	> 5000	[55179-31-2]
Metiram	49	5.2	-	-	-	> 2000	[9006-42-2]
Thiram	47	2.9	1.0 (1990)	-	-	> 1000	[137-26-8]
Herbicides							
Simazin	83	1.2	-	-	-	> 3100	[122-34-9]
Paraquat	68	1.6	0.5 (1975)[F] 0.1 (1975)[G]	0.1	0.001	236-500[I]	[1910-42-5]
Glyphosate	45	1.2	-	-	-	> 5000[I]	[1071-83-6]
Insecticides							
Azinphos-methyl	47	1.8	0.2 (1986)[Skin]	0.2	0.0025	220	[86-50-0]
Acaricides							
Bromopropylate	60	1.5	-	-	0.008	> 4000	[18181-80-1]
Hexythiazox	47	1.1	-	-	-	> 5000	[78587-05-0]

[A]Threshold Limit Value (mg m^{-3}), Time-Weighted Average (TWA) with year of adoption (ACGIH, 1992); [B]Maximal Accepted Concentration (MAC) mg m^{-3}, Arbeidsinspectie (1994); [C]Accepted Daily Intake ADI in mg kg^{-1}, (Worthing, 1987); [D]Acute percutaneous LD_{50} for rat mg kg^{-1}; [E]CAS, registration number of Chemical Abstracts Service; [F]for total dust; [G]for inspirable fraction; [Skin]refers to the potential contribution to the overall exposure by the cutaneous route including mucous membranes and eyes, either by airborne or, more particularly, by direct contact with the substance (ACGIH, 1992); [H]ADI calculated by EEC (WHO, 1990); [I]for rabbit mg kg^{-1}.

Figure 1 Outline of the study[4]



[4] THPI = tetrahydrophtalimide, metabolite of captan
Residential exposure = exposure inside homes of fruit growers

CHAPTER 2

Exposure to pesticides in fruit growing[1]

ABSTRACT

Large scale exposure surveys in fruit growing in The Netherlands were carried out during application of pesticides among 94 farmers and during re-entry tasks among 138 persons working in orchards. For 61 workers repeated measurements on the same subject were obtained. Additionally, pesticide contamination levels inside the homes of 10 fruit growers were measured.

In this study exposure to pesticides has been described in terms of average exposure levels and variability in measured concentrations. The relative contribution of the respiratory route and different skin sites to total exposure has been assessed. Captan was used as a marker for exposure. Also, total exposure for the fruit grower and his family members during the growing and harvesting season was estimated.

Inhalable dust exposure was measured with a personal air sampler and dermal exposure with skin pads and hand rinsing. Dislodgeable foliar residue (DFR) was measured by taking leaf punches from foliage in the orchards.

Between respiratory exposure and dermal exposure of hands, wrists and forehead, differences were observed between several tasks. Grouping workers according to performed tasks, therefore will depend on the exposure measure(s) (*e.g.* hands, forehead,

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inhalable dust), which are considered to be relevant for a specific study purpose. As exposure per unit of time was in the same order of magnitude for different tasks, individual time spent on these tasks is crucial for estimating total exposure.

In general, within-worker variability of all exposure measures was larger relative to between-worker variability. Therefore, repeated measurements are necessary to estimate individual exposure accurately. Variability in dermal exposure on the same body location was small relative to variability between different body locations.

Differences in total exposure, including exposure inside home, between the fruit grower and the son were small. Exposure of the wife was around 2-3 times lower than for the fruit grower and the son. To estimate internal exposure dose for epidemiological purposes and risk assessment, the key element is ascertaining accurate information on the contribution of the specific exposure routes to the effective target dose.

INTRODUCTION

A general lack of information exists on the incidence of adverse chronic health effects due to human pesticide exposure. A workshop on potential chronic health effects of pesticides in Colorado (1988) concluded that there is some concern that chronic adverse health effects in humans might result from excessive pesticide exposure that could occur in occupational settings. The fact that very few epidemiological studies have been conducted in this area was partly attributed to difficulties in identifying and quantifying exposures to different pesticides over time and the existence of many potential confounding factors (Baker *et al.* 1990).

Although pesticides in general are absorbed more efficiently through the lungs than through the skin (Durham and Wolfe 1962), the dermal route predominates under many circumstances (Coutts 1980, Stevens & Davis 1981, WHO 1982). The evaluation of occupational exposure to pesticides of agricultural workers is an integral part of the risk assessment process, but at present there is no internationally accepted, harmonized approach (Chester 1993).

A review of strategies to determine occupational exposures in risk assessment is given by Teschke *et al.* (1994). Methods range from: 1) predictive exposure models, based on mathematical representations and population behaviour patterns, which may be based on worst case scenarios; 2) Use of existing data as a 'surrogate', assuming that exposure is determined more by the physical properties of the formulation and the methods and conditions of use than by the chemical nature of the pesticide (this, has not been validated); 3) Measurements in quasi-experimental situations, which has been the recommended method for assessing occupational exposure to pesticides for risk assessment in the registration process; 4) Measurements on a representative sample of the exposed population, which is seen as the 'gold standard' of exposure assessment strategies. As main difficulties of this last approach practicability and costs are mentioned, but as advantage that unbiased estimates of exposure may be obtained.

Data on exposures to pesticides have been collected over many years. Difficulties in comparing exposure data are due to differences in physicochemical properties of the pesticides, used exposure measurements methods, the study purposes (registration *vs* health effects), type of farming (indoor *vs* outdoor), used equipment, and differences in meteorological conditions between countries. Studies tend to contain small data sets and furthermore part of the data concern unpublished data submitted in the framework of registration procedures. Recently, exposure data and published proposals for generic data bases were reviewed (van Hemmen, 1993). It was concluded that these data bases can be used as a first step for risk assessment.

Most studies on exposure to pesticides in The Netherlands, concern indoor studies in greenhouses for flower growing, usually on year-round culture basis (Boleij *et al.*, 1991; Brouwer, D. *et al.*, 1992; Brouwer, R. *et al.*, 1992, 1993). Little data exists on outdoor occupational exposure to pesticides. Outdoors, in fruit growing, over 30 different pesticides are used (Table 1) of which fungicides are the most frequently used throughout the spraying season. Most insecticides and acaricides are used for pest control only when necessary. Herbicides are only used once or twice early in the spraying season. Pesticide use typically concerns mixed exposures, fluctuating over the year.

Table 1 Top 10-pesticides in fruit growing in 1989, according to percentage of farms (N = 915), and mean number of sprayings per year, The Netherlands

Pesticide	Farms(%)	No. of sprayings/y
Fungicides		
Captan	94	9.6
Bitertanol	66	3.1
Metiram	49	5.2
Thiram	47	2.9
Herbicides		
Simazin	83	1.2
Paraquat	68	1.6
Glyphosate	45	1.2
Insecticides		
Azinphos-methyl	47	1.8
Acaricides		
Bromopropylate	60	1.5
Hexythiazox	47	1.1

Data on repeated measurements of exposure to pesticides in agriculture are scarce, especially for dermal exposure. In general, both environmental and production factors are recognized to influence the day-to-day variability in exposure and to a somewhat lesser extent also the between-worker component of variance. It was concluded that in situations where workers work outdoors in an intermittent process a 4 - 5 fold increase in number of repeated measurements will be needed to provide the same precision of the average exposure, compared with a situation where workers are indoors in a continuous process (Kromhout, 1994).

During application of pesticides and re-entry work in treated orchards, data on dermal and respiratory exposure were obtained in a large scale study from 94 farmers during application in 1990 and 138 workers during re-entry in 1991 and 1992. For 61 workers repeated measurements on the same subject were obtained. Additionally, pesticide contamination level inside the homes of 10 fruit growers was measured. Because fungicides are most frequently used during the spraying season, captan as the most representative of the wettable powder formulations, was used as a marker for exposure. Emphasis in this article is put on a description of average exposure level and variability in exposure. Both dermal and respiratory exposure for the main tasks and main determinants are described. Also within- and between worker variability of exposure was studied, and for dermal exposure, variability of exposure between body sites. Finally, an estimation of total exposure is made for the fruit grower and his family members during the growing and harvesting season.

MATERIALS AND METHODS

Fruit growing

The fruit growing sector in The Netherlands consists of around 3000 fruit growing farms. Apples and pears constitute respectively 70% and 22% of the total crop area of 24.000 hectares. Pesticides are regularly applied from March till November. Nowadays the modern standard tree orchard has 2000 - 3000 trees per hectare with a mean tree height of around 2 - 2.5 meters. The traditional old bush tree orchards with a tree density of less than 1000 per hectare and a tree height of over 3 meters have almost disappeared. Airblast sprayers towed behind a tractor are predominantly used for application of pesticides. For 57% of the farms, fruit growing is the only activity and the main sideline activity for 38% is arable farming. Over 6,500 people are occupationally exposed to pesticides in fruit growing in The Netherlands. Fruit growing is typically a family business and therefore exposure is not only limited to the fruit grower. Family members like the farmers' wife and their children often participate in work in the orchards. During harvesting (August - October) also hired seasonal workers, may be exposed to pesticide residues during re-entry of the crop.

Labour is characterized by seasonal variations in performed tasks. Application of pesticides consists of diluting, mixing, loading, spraying, and maintenance and cleaning of used equipment. These tasks are mostly performed by the farm owner and sometimes by one of his sons. During summer other tasks performed in the orchards are bending of branches, thinning of fruit and summer pruning. From August till October harvesting, sorting and transport of fruit take place, which often requires extra labour force. In winter planting and treatment against cankerous growth in trees are the main tasks in the orchards.

In fruit growing in The Netherlands, traditionally, the work environment is close to the residential environment. Often the orchards surround the house. Sources of exposure to pesticides, therefore, can be divided into sources connected to the work environment during the application of pesticides, re-entry tasks in the orchards, and sources of pesticide residues within the residential environment. Pesticides may be transported from the orchards into the house via air or through contaminated clothing or by contaminated people or pets. Individual exposure is determined by exposure rate of tasks performed and duration spent on each task. Time spent in the residential environment is greater when compared with work in the orchards, and so contamination of the residential environment may contribute substantially to total exposure of the fruit growers family.

Selection of Fruit Farms

In 1989 a postal questionnaire was anonymously sent to all 2,730 members of the Dutch National Fruit Growers' Organization (NFO). Basic information was gathered on orchard type, population, equipment used, performed tasks, pesticides used, duration of treatments and frequency of pesticide use. 1121 respondents (41%), filled out the questionnaire. Complete information on all items was available from 915 questionnaires. The initial population for further studies consisted of 447 respondents who were willing to participate in future surveys. A telephone interview among 141 randomly selected non-respondents revealed that 45% thought the questionnaire was not applicable to their situation. Reasons mentioned were a small crop area (17%), fruit growing was a sideline (15%) or because termination of farm business had already been planned (13%). Further reasons were the high number of questionnaires that had to be returned (26%) or expressing no interest in the subject (18%).

For the exposure surveys fruit growers were randomly selected from the population of 447 farmers from the three main fruit growing regions, South-West ('Zeeland'), Middle ('Betuwe') and the polder area ('Flevopolders'). Actual participation depended on a combination of performed activities on the farm, and weather conditions on days when surveys were carried out.

Sampling and analysis of captan

During field work checklists were used to register auxiliary data on characteristics of the farm, tasks and used equipment and on used amounts of pesticides. During re-entry studies farm owners also kept a diary of performed sprayings.

The PAS-6 sampling head was used for sampling inhalable pesticide particles (Boleij *et al.*, 1995). The PAS-6 was equipped with a glassfibre filter (diameter 2.5 cm Whatman, GF/A), connected to a portable pump device (DuPont P2500) with a flow of 2 L/min.

To measure dermal exposure, skin pads made of α-cellulose (Schleicher and Schüell), diameter 3.5 cm were used and placed between two circular Fixomull stretch bandage plasters (BDF), diameter 5 cm, with a circular opening of 2.5 cm in the upper plaster layer. Body locations for the skin pads were based on the standard WHO-protocol (WHO, 1982,1986) and in some studies additional locations were used. Pads, were placed on the forehead, inner side of the wrists (left, right), on the back between shoulder blades, the front ("V"), on the back of the neck, and on the forearm (upper surface, midway between elbow and wrists, at the left side for a right-handed person). Pads were applied on clothing, if worn, or directly on the skin. For each person performing re-entry tasks, both hands were rinsed once each day. Each hand was put in a polyethylene sandwich-bag and closed around the wrist with a rubber band. Through a funnel 150 ml ethanol (50%) was added while keeping the fists clenched to prevent early contact with ethanol. Then, the hands were rinsed during 30 seconds by alternately moving both sides of the spread hands over a table surface. The solution was put into a polyethylene vial and stored in a cooler. The samples were analyzed in the laboratory on the same day.

Dislodgeable foliar residue (DFR) was measured by taking leaf punches (ø 25 mm) in duplicate with a leaf puncher connected to a glass vial. One leaf sample consisted of 40 punches from 8 different trees. Analysis of skin pads, air samples, hand rinse samples and leaf punches was carried out using high performance liquid chromatography (HPLC). Before analysis, samples were stored at -30 °C. The following chemicals were used: ethanol absolut (p.a.) (Merck, Amsterdam, The Netherlands); methanol for HPLC; acetonitrile for HPLC (Janssen Chimica); water (nanopure); Dioctyl-sulfosuccinate; sodium salt (96%) (SurTen) (Aldrich).

A punch (16 mm diameter) from the α-cellulose skin pad was put into a glass vial (5 ml) with tweezers. An acetonitrile/water mixture (50/50%) was added with a dispenser of 1.5 ml. The vial was closed with a cap and vibrated in an ultrasonic bath for 5 minutes. Next, the sample was put into a HPLC vial and placed into the HPLC autosampler at 4 °C. The air sample filter was put into a glass vial (5 ml) with tweezers. An acetonitrile/water mixture (50/50%) was added with a dispenser of 1.5 ml. The vial was closed with a cap and vibrated in an ultrasonic bath for 5 minutes. The sample was put through a syringe with 0.2 μm filter (Schleicher and Schüell, Spartan 13) into a HPLC vial and placed into the HPLC autosampler at 4 °C. A few millilitres of the hand rinse sample were put into a glass vial (5 ml). The vial was closed with a cap and vibrated in an ultrasonic bath for 5 minutes. Next, the sample was put through a syringe with 0.2 μm filter (Schleicher and Schüell, Spartan 13) into a HPLC vial and placed into the HPLC autosampler at 4 °C. Then, 50 ml demineralized water and 4 drops SurTen-solution were added into a glass vial (100 ml), containing the leaf punches. The vial was closed with parafilm and a screw-cap and shaken for 15 minutes. Two ml of sample and 2 ml ethanol (absolut) was put into a glass vial (5 ml) with a pipette. The vial was closed with a cap and vibrated in an ultrasonic bath for 5 minutes. The sample was put through a syringe with 0.2 μm filter (Schleicher and Schüell, Spartan 13) into a HPLC vial and placed into the HPLC autosampler at 4 °C.

The HPLC-system consisted of a Spectra-Physics Sp 8800 Ternary gradient system with a flow of 0.5 ml/minute and helium degassing; Spectra-Physics autosampler SP8875 with 20 μl sample loop and autosampler cooler SP8760; Chrompack 2 × 10 cm, 3 mm internal diameter C18 reversed phase column, particle size 5 μm, with a 1 cm guard column; Spectraflow 757 UV absorbance detector, Kratos Analytical Instruments with a wavelength of 210 nm; Spectra-Physics SP 4270 integrator connected to Epson-XT work station with Winner/286 and Labnet software for data storage and manipulation; Eluent: acetonitrile, water (nanopure) and methanol after ultrasonic degassing for 5 minutes. The limit of detection was 17 μg/L, which is equivalent to 12.8 ng cm^{-2}.

Measurement strategy

Exposure surveys were carried out during the application of captan in orchards by fruit growers (from 30 May - 24 October 1990) and during other work activities in the orchards (re-entry tasks) by fruit growers, family members and seasonal workers from 5 August - 24 October 1991 and from 11 June - 8 September 1992, respectively. In 1990 exposure measurements during application among 94 farmers on captan were carried out, four of which were repeated measurements on the same person. Repeated measurements were available for re-entry tasks for 57 persons out of a population of 138 persons. Average duration of activities was 2 hours for application of captan (standard deviation 1 hour, range 22 min - 4.8 hours), and 6.5 hours (standard deviation 2.3 hours, range 55 min - 10 hours) for re-entry tasks. Mean age of the persons was 43.2 years (range: 16-69) in 1990 and 38.0 years (range: 15-82) and 31.6 years (range: 14-80) for 1991 and 1992, respectively. As generally no differences were observed during application or re-entry between the exposure of the left and right hand, the average of both hands was used.

Also the contribution of residential exposure was assessed. Exposure and contamination levels measured inside homes (n = 10) was based on a small number of personal measurements of wives not actively participating in work in the orchards at that time. Measurements took place during the harvesting season, within two days after a spraying was performed, which was considered a worst-case scenario for contamination of the residential environment. Samples of the floor were taken with a sampler connected to a vacuum cleaner.

Statistical Analysis

All data analyses were performed using Statistical Analysis System Software (SAS). The exposure distribution was studied using PROC UNIVARIATE. PROC TTEST was used to compare average exposure of subgroups. A two-sided *P*-value of 0.05 was used as criterion for statistical significance. A random-effect's ANOVA model was used to estimate between- and within-worker variance components. Variability of dermal exposure between body locations was also assessed. All variance components were estimated with PROC NESTED. The range ratio of the between-worker distribution (${}_{b}\hat{R}_{0.95}$) was calculated as quantitative measure of uniformity of exposure within occupational groups (Rappaport, 1991).

RESULTS

Main type of farming, according to the postal questionnaire (N = 915) consisted of hard fruit. Apples were grown on 93% of the fruit farms, with a mean crop area of 7.1 hectares, pears 69% (2.6 hectares), plums 26% (0.5 hectares) and cherries 7% (1.0 hectares) and other crops 1% (1.7 hectares). All observations on application of captan in 1990 concerned the use of airblast sprayers pulled by a tractor. A cabin on the tractor was used by 52% of the farmers. The main type of sprayer was a conventional sprayer (60%), 37% of the farmers used a modern 'cross-current' sprayer and 3% a 'tower-type' sprayer.

Application

Exposure data for the application of pesticides are presented in Table 2. Highest dermal exposure to captan was found on arms and wrists. In Table 3 data on exposure during application of captan are compared for fruit growers according to cabin use. For respiratory exposure no significant difference was observed with the presence of a cabin, while its presence was clearly related to lower dermal exposure varying from a 6.7 times lower exposure of the neck to 27 times lower exposure of the forehead. For spraying, repeated measurements were available for only four workers. Both variability within and between fruit growers were large (data not shown).

During preparation of tank mixtures, exposure on the forehead (n = 10) was 6.9 mg m^{-2} hr^{-1} (AM) with a range of 0.70-48.2, and on the wrists (n = 23) 71.0 mg m^{-2} hr^{-1} (AM) (range 0.75-837) (data not shown).

Re-entry

Exposure data for the main activities in fruit growing during re-entry tasks in 1991 and 1992 are presented in Table 4. All exposure measures had a range ratio (${}_{b}\hat{R}_{0.95}$) larger than two, indicating non-uniformity in exposure between workers. In general, within-worker variability of all exposure measures was larger relative to between-worker variability (median ${}_{w}S_{g}$ = 3.2 *vs* median ${}_{b}S_{g}$ = 1.9). Highest day-to-day variability was found for respiratory exposure and exposure of the hands. Highest dermal exposure was found on the hands and wrists during re-entry tasks.

Table 2 Exposure during application (comprising mixing, loading and spraying), fruit growing, The Netherlands (1990)

	N	AM	GM	GSD	Range
Respiratory exposure ($\mu g\ m^{-3}$)					
Inhalable fraction	94	81.6	22.6	5.2	0.26-744
Dermal ($mg\ m^{-2}\ hr^{-1}$)					
Wrists	98	10.3	3.5	4.5	0.17-536
Arm	89	22.0	3.6	7.3	0.07-303
Forehead (front)	66	6.15	1.2	6.6	0.05-178
Neck (back)	73	2.06	0.8	3.9	0.04-76.3

N = number of observations; AM = maximum likelihood arithmetic mean; GM = geometric mean; GSD = geometric standard deviation; Range = range of arithmetic means.

Table 3 Exposure during application of captan according to the use of a cabin on the tractor, fruit growing, The Netherlands (1990)

	No Cabin			Cabin			
	N	AM	GSD	N	AM	GSD	P-value[A]
Respiratory exposure ($\mu g\ m^{-3}$)							
Inhalable fraction	46	62.4	4.2	48	101.6	6.3	0.85
Dermal exposure ($mg\ m^{-2}\ h^{-1}$)							
Wrists	46	10.4	3.3	52	8.55[B]	5.3	0.007
Arm	44	48.6	5.9	45	3.27	4.4	< 0.001
Forehead	38	12.9	5.5	28	0.47	2.7	< 0.001
Neck (back)	40	3.69	3.7	33	0.55	2.5	< 0.001

N = number of observations; AM = maximum likelihood arithmetic mean; GSD = geometric standard deviation; [A]*P*-value: *t* test; [B]not log normally distributed.

Table 4 Exposure during re-entry of orchards to captan, fruit growing, The Netherlands (1991 and 1992)*

	N	K	AM	GM	GSD	${}_b\hat{R}_{0.95}$	${}_w\hat{R}_{0.95}$	Range
<u>Respiratory exposure</u> ($\mu g\ m^{-3}$)								
Inhalable fraction	154	108	49.8	14.0	5.1	3.1	541	0.17 - 547
<u>Dermal</u> ($mg\ m^{-2}\ hr^{-1}$)								
Wrist	188	133	4.1	1.47	4.3	17.3	143	0.04 - 26.4
Hand rinsing[A]	182	128	21.1	7.7	4.2	45.1	65.3	0.03 - 86.5
Forehead (front)	181	131	0.3	0.13	4.1	17.9	108	0.02 - 17.3
Arm	176	127	2.6	1.14	3.7	14.2	80.8	0.02 - 19.9
Sternal area	184	131	0.1	0.06	2.3	7.1	12.9	0.02 - 1.24

*Exposure variables were not log normally distributed for combined data of 1991 and 1992; [A]assuming a skin area of stretched palms and fingers of 360 cm^2 according to Fiserova-Bergerova (1993); N = number of observations; K = number of farm workers; AM = maximum likelihood arithmetic mean; GM = geometric mean; GSD = geometric standard deviation; ${}_b\hat{R}_{0.95}$ = ratio of 97.5th and 2.5th percentiles of the between-worker distribution; ${}_w\hat{R}_{0.95}$ = ratio of 97.5th and 2.5th percentiles of the within-worker distribution; Range = range of arithmetic means.

Table 5 Exposure during re-entry of orchards treated with captan, according to tasks, fruit growing, The Netherlands (1991, 1992)

	Thinning			Bending/Tying up			Pruning			Harvesting			
	N	AM	GSD	N	AM	GSD	N	AM	GSD	N	AM	GSD	remarks‡
<u>Respiratory exposure</u> ($\mu g\ m^{-3}$)													
Inhalable fraction	7	17.5	2.3	36	19.3	4.9	34	51.6	5.6	68	55.8	4.1	A, B
<u>Dermal exposure</u> ($mg\ m^{-2}\ h^{-1}$)													
Wrists	11	2.83	1.8	35	2.0	2.9	35	1.96	4.0	94	6.16*	5.1	C, D, E
Hand rinsing	10	37.5	2.8	36	20.1	3.3	36	16.2	3.9	94	19.0	4.5	F, G, H
Arm	9	1.78	1.9	34	1.55	2.9	32	2.0	4.0	90	3.06*	4.0	
Forehead	10	0.19	2.8	33	0.17*	3.4	34	0.16*	2.9	92	0.45*	4.5	I
Sternal area	10	0.27*	4.0	34	0.08*	2.0	34	0.06*	1.6	94	0.09*	2.4	

* Not log normally distributed; ‡ significantly different exposure between tasks (*P*-value: *t* test); N = number of observations; AM = maximum likelihood arithmetic mean; GSD = geometric standard deviation.

A bending/tying up and pruning ($P=0.05$); B bending/typing up and harvesting ($P<0.01$)
C thinning and bending/tying up ($P<0.01$); D thinning and pruning ($P<0.01$); E pruning and harvesting ($P=0.01$)
F thinning and bending/tying up ($P=0.05$); G thinning and pruning ($P=0.01$); H thinning and harvesting ($P<0.01$)
I pruning and harvesting ($P=0.04$).

In Table 5 results for specific re-entry tasks in treated orchards are given. Inhalable dust exposure (AM) during bending/tying up was significantly lower (19.3 $\mu g\ m^{-3}$) than during pruning (51.6 $\mu g\ m^{-3}$) and harvesting (55.8 $\mu g\ m^{-3}$). Differences were also observed for dermal exposure. For thinning, exposure of the wrists was higher compared with bending/tying up, and pruning. Also exposure of wrists was higher for harvesting than for pruning. For hand rinsing, exposure was higher for thinning as compared with other categories of re-entry activities. Exposure of the forehead was significantly higher during harvesting as compared with pruning. For exposure of arms and sternal area no differences between tasks were observed.

Comparison between application and re-entry

Respiratory exposure was statistically significant (*t* test: P=0.03) higher during spraying (AM = 81.6 $\mu g\ m^{-3}$) when compared with re-entry tasks (AM = 49.8 $\mu g\ m^{-3}$). Dermal exposure was higher during application as compared with re-entry for the corresponding body locations wrists, arm and forehead, expressed per unit of time (in each case *t* test: $P<0.01$).

Uniformity of dermal exposure

For dermal exposure during re-entry, variability is presented in Table 6 according to variance between workers, between performed tasks, between body locations (pads) and within locations. When all skin pads were taken into account, body location explained the major part of total variability ($_{bl}S_g$ = 4.5), relative to within-location ($_{w}S_g$ = 2.8) and between-task variability ($_{bt}S_g$ = 1.4). No variability was observed in average dermal exposure between workers. No differences between forehead and sternal pad were found ($_{bl}S_g$). Also no differences between pads at the same body site were found for arm pads in duplicate and rinsing of both hands and for pads at left and right wrist.

Table 6 Variance between workers, between tasks, between and within locations of dermal exposure measurement, re-entry (1991,1992)

Location	Method	N	K	Total $({}_{t}S_{g})$	Between Worker $({}_{bw}S_{g})$	Between Task $({}_{bt}S_{g})$	Between Location $({}_{bl}S_{g})$	Within Location $({}_{w}S_{g})$
Head, Sternal, Wrists*, Arm	pad	677	126	6.4	1	1.4	4.5	2.8
Forehead, Sternal area	pad	339	126	3.4	2.0	1	1	2.8
Arm, 2 pads on same arm	pad	292	126	5.0	1	3.5	1	2.7
Hands (left, right)	hand rinsing	348	126	5.7	2.5	2.6	1	3.1
Wrists (left, right)	pad	167	126	5.8	1.6	3.6	1	3.0

* average of left and right hand; N = number of observations; K = number of farm workers; ${}_{t}S_{g}$ = geometric standard deviation of the total distribution; ${}_{bw}S_{g}$ = geometric standard deviation for the between-worker distribution; ${}_{bt}S_{g}$ = geometric standard deviation for the between-task distribution; ${}_{bl}S_{g}$ = geometric standard deviation for the between-body location distribution; ${}_{w}S_{g}$ = geometric standard deviation for the within-location distribution.

Total exposure

In all homes (n = 10) captan could be detected on floors (average 7.6 $\mu g\ m^{-2}$; range 0.91 - 14.9). Exposure of hands, from hand rinsing, varied between 0 and 0.89 mg $m^{-2}\ hr^{-1}$ (n = 4; average 0.27) and inhalable dust concentration varied from 0 - 0.47 mg m^{-3} (n = 3, average 0.3). The average sampling duration for hand exposure was 133 minutes and for inhalable dust exposure 363 minutes. Contamination level, measured in the kitchen on the table, sink unit, and window-sill varied from 0 - 476 $\mu g\ m^{-2}$ (n = 16). This is very low compared with dislodgeable foliar residue in the orchard of around 20.000 $\mu g\ m^{-2}$.

As an example, total seasonal exposure to captan is estimated for the fruit grower, for his son, assisting on the application and re-entry tasks, and for the fruit grower's wife, who performs part time re-entry tasks. Total exposure was calculated for inhalable dust exposure and dermal exposure of the hands, representing skin sites which may be actively contaminated during work, and exposure of the forehead, as an example of a skin site which is contaminated more passively or indirectly. Exposure by ingestion was not allowed for, since no feasible measurement methods were available and generally contribution of occupational exposure by ingestion is considered of minor importance. Total exposure was calculated for the growing season and harvesting season separately, assuming equal exposure rates. Total exposure was calculated by multiplying exposure rate with distributions of duration spent on different tasks (Annex 1). No detailed information on time spent on specific tasks for the study population was available, therefore, the typical duration of the different tasks was taken. In Table 7 exposure contribution (%) of main tasks is given together with the total cumulative amount (mg).

In general total respiratory and dermal exposure was higher during the harvesting season, since duration of time spent on these tasks predominates. Differences in exposure between the fruit grower and the son were small. Exposure of the wife was around 2-3 times lower than for the fruit grower and the son. The effect of a cabin during application was only apparent for dermal exposure of the forehead.

Table 7 Relative contribution of main tasks to total exposure per route to captan for fruit grower, son and wife, according to season

	Respiratory route			Dermal route					
	Inhalable dust			Wrists			Forehead		
	Fruit grower	Son	Wife	Fruit grower	Son	Wife	Fruit grower	Son	Wife
Growing season: May - July									
Application[A]	4%	1%	0%	5 / 6%	1 / 2%	0	4 / 54%	1 / 23%	0%
Re-entry	95%	98%	91%	84%	87%	47%	46-96%	77-99%	100%
Inside Home	1%	1%	9%	10%	11%	53%	-[B]	-	-
Total amount (mg) *	36.6	35.4	6.4	202	194	60	11.8/24.6	11.4/14.6	1.9
Harvesting season: August - October									
Application[A]	2%	< 1%	0	2 / 3%	<1/ <1%	0	2 / 32%	1 / 9%	0%
Re-entry	97%	99%	98%	89%	93%	84%	68-98%	91-99%	100%
Inside Home	< 1%	< 1%	2%	7%	5%	17%	-	-	-
Total amount (mg) *	44.3	52.3	26.4	238	274	151	14.3/20.7	16.9/18.5	8.5
Total Season: May - October									
Total amount (mg) *	81	88	33	440	468	211	26/45	28/33	10

* Exposure dose based on a ventilation rate of 20 L/min for respiratory exposure and a skin area of 720 cm^2 for the hands and 650 cm^2 for the forehead;
[A] dermal exposure is given for spraying with and without a cabin on the tractor, respectively; [B] not measured.

DISCUSSION AND CONCLUSIONS

Average respiratory exposure to captan ranged from 0.17-744 $\mu g\ m^{-3}$, and was clearly below the Dutch maximum accepted concentration (MAC) of 5 mg m^{-3} (ISZW, 1995) for all tasks. Dermal exposure ranged from 0.02-536 mg m^{-2} hr^{-1} with clear differences between body locations. Higher respiratory exposure, as found for application compared with re-entry of orchards treated with captan, may be due to a contribution of exposure to undiluted captan dust during preparation of tank mixtures. Differences in respiratory exposure between different re-entry tasks may be caused by a combination of the tasks performed and residue level of captan in orchards.

Dermal exposure per unit of time for wrists, arms and forehead was higher during spraying compared with re-entry. However, average dermal exposure duration of application was much shorter (2 hr) than for re-entry (6.5 hr) and also less frequent (approximately 10 sprayings per year for captan). For many workers re-entry concerns work that is performed on a daily basis. Also exposure of wrists and forehead during spraying with a cabin on the tractor was higher as compared with re-entry tasks in orchards. This may partly be due to exposure that occurs during mixing and loading, since dermal exposure per unit of time was highest during preparation of tank mixtures. As mixing and loading normally takes place at the beginning of a work cycle, skin contamination from preparation tank mixtures will potentially be available for uptake for a long period of time. The hands may also form a secondary source of contamination for other parts of the skin or may cause oral uptake.

Variability in exposure may be explained by changes in work practice, work style and personal hygiene, and changes in weather conditions. Total variability in exposure was large for application of captan (GSD 3.9 - 7.3). Although the number of repeated measurements on the same person was small, both variability within and between workers seemed to be large. For re-entry, total variance was smaller than for application (GSD 2.3 - 5.1). Persons were not uniformly exposed for any of the exposure measures. For dermal exposure 95% of individual mean exposures were in a range of 7 - 45 fold, while for respiratory exposure this range was only 3-fold.

No differences were found in dermal exposure for arms and sternal area between thinning, bending/tying up, pruning and harvesting. Between respiratory exposure and dermal exposure of hands, wrists and forehead, however, differences were observed between several tasks. Grouping workers according to performed tasks, therefore will depend on the exposure measure(s) (*e.g.* hands, forehead, inhalable dust), which are considered to be relevant for a specific study purpose.

As variability of exposure between skin areas at the same body locations (*e.g.* pads on the arm) was small relative to day-to-day variability within the same body locations, it seems unnecessary to measure both hands or different sites of the same body location. Variance between different body locations, however, was large and not uniform relative tot day-to-day variation within body locations. Repeated measurements are required, since for most dermal sites day-to-day variability was most prominent.

Captan, as most frequently used fungicide, was used as marker for exposure. Captan may be considered a good surrogate for potential external exposure to powdery fungicides under these circumstances. However, for exposure during re-entry of the orchards, results from a study on one specific pesticide should not be generalized to other pesticides and/or other situations, unless data on decay under the specific environmental conditions are available.

Exposure to pesticides in fruit growing typically concerns mixed exposure to a large variety of pesticides and chemical additives. It is likely that a mutual dependency exists on a farm in exposure rates between application, re-entry and contamination level in the residential environment. Outside the growing and harvesting season (November - March), exposure levels are assumed to be lower. The assumption of equal exposure for the growing and harvesting season is a simplification, since spraying frequency and amount of pesticides applied for both seasons may vary. Differences in respiratory and dermal exposure levels between application and re-entry were, although significantly different, not very large. As time spent on re-entry work is longer than for application, highest relative contribution to a person's exposure was on average found for re-entry. Time spent on different tasks, however, may be highly variable between persons, over the year. The maximal difference found in cumulative dose for the forehead of a factor between the wife and the fruit grower, factor 4.5 (45/10) was not large. It should be noted here that peak exposures during mixing and loading are not considered separately. Peak exposures may cause adverse effects, that may be distinct from those of cumulative exposure (Stewart, 1991). This may be important when considering cumulative exposure of the fruit grower, who prepares the tank mixtures, compared with workers only involved in re-entry tasks.

Calculation of the contribution of residential contamination to total exposure was based on relatively short sampling durations as compared with total time spent inside homes, which may have biased our estimates. For personal dermal exposure of the hands inside the house, it cannot be ruled out that part of measured exposure was due to tasks performed by the person in the orchards earlier. Although persons had washed their hands since the last time they were in the orchards, this may not have been completely effective. Potential exposure inside homes was lower as compared with exposure in the orchards, but residential exposure concerns a general population, including young children and elderly who may be more sensitive, and who will spend relatively more time inside or around the house.

Additionally, the assumption that ingestion only will be of minor importance on total occupational exposure is questionable, particularly when part of the exposure takes place in the home environment. Residential exposure and its contribution to total occupational exposure may influence epidemiological exposure-response analyses. Despite the low number of measurements in the home environment, it is clear that dependent on personal work activities and time spent inside home, exposure sources other than application should not be neglected.

As stated by Loomis and Savitz (1994) failure to include all exposure sources can bias results of epidemiological studies. Unmeasured exposure, for instance from exposure sources in the residential environment due to pesticide use in the orchards, is not likely to be a constant proportion of the measured exposure. Therefore, it is impossible to predict if dose-response relationships will be biased in an upward or downward direction. More attention should be given to all sources and routes of exposure in situations where work and residential environment are closely connected to make valid estimations of exposure for epidemiological purposes. Furthermore, setting standards for occupational exposure levels for respiratory exposure are not applicable in situations where other exposure routes may be predominant.

Many studies in recent years have indicated that dermal exposure is not constant over body area due to factors about the work place, personal behaviour and hygiene (Fenske 1990, 1993). As variation in permeability of the skin depends on both the physicochemical properties and the skin site exposed, more should be known about the variability in exposure of the different measured skin locations and factors affecting uptake and metabolism, before total dermal uptake can be estimated. The relevant body locations for uptake need not to be the highest exposed skin area. This was recently illustrated by a study on the relation between dermal and respiratory exposure to captan and the metabolite THPI in urine of fruit growers. A clear relationship was found between skin exposure of neck and ankles with THPI in urine (Cock *et al.*, 1995b). No relation was found for respiratory exposure or total dermal exposure, calculated from measured exposure on skin pads according to models described in the literature. From this perspective total dermal exposure is an imperfect and potentially biased estimate of internal dose.

In epidemiological studies a valid estimate of exposure dose may be complicated by differences in effect of repeated exposure to relatively low levels of exposure during re-entry as compared with infrequent exposure to high levels as for instance during mixing and loading. Standards for (respiratory) exposure are based on a measurement duration of 8 hours. For dermal exposure the biologically relevant period however may be longer.

In conclusion, to estimate internal exposure dose for epidemiological purposes and risk assessment, the key element is ascertaining accurate information on the contribution of the specific exposure routes to the effective target dose. In this respect, future attention should concern the role of variation in dermal uptake according to the exposed body sites.

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Annex 1 Exposure to captan, and time spent on work, in home environment and elsewhere of fruit grower, son and wife, according to season

	Exposure			Cumulative duration (hours)[A]		
	Inhalable dust ($\mu g\ m^{-3}$)	Wrists ($mg\ m^{-2}\ hr^{-1}$)	Forehead ($mg\ m^{-2}\ hr^{-1}$)	Fruit grower	Son	Wife
Growing season: May - July						
Application[B]	82	8.6/10.4	0.47/12.9	16	4	0
Re-entry	50	4.1	0.30	576 (12×48)[D]	576 (12×48)	96 (12×8)
Inside Home	0.3	0.27[E]	-[C]	1080 (12×90)	1080 (12×90)	1620 (12×135)
Elsewhere	0	0	0	488 (12×41)	488 (12×42)	444 (12×37)
Harvesting season: August - October						
Application		*as growing season*		8	2	0
Re-entry				720 (12×60)	864 (12×72)	432 (12×36)
Inside Home				900 (12×75)	720 ((12×60)	1284 (12×107)
Elsewhere				532 (12×44)	574 (12×48)	444 (12×37)

[A] Cumulative duration 3 months × 30 days × 24 hr = 2160 hours

[B] Dermal exposure is given for spraying with and without a cabin on the tractor, respectively

[C] Not measured

[D] Number of weeks, and hours per week between brackets

[E] Calculated from hand rinsing exposure ($mg\ hr^{-1}$).

CHAPTER 3

Determinants of exposure to pesticides in fruit growing[1]

ABSTRACT

A series of studies has been carried out on occupational exposure to pesticides among fruit growers in The Netherlands during spraying and re-entry of the crops between 1990 and 1992 to quantify exposure and identify determinants of exposure. Determinants of exposure are discussed as a starting point for hazard identification and control *i.e.* measures to be taken to reduce occupational exposure to pesticides.

Cabin use on the tractor was the most prominent determinant of dermal exposure during spraying. Dermal exposure studied in subgroups according to cabin use was related to determinants of exposure, which varied for different body sites. For respiratory exposure, factors related to preparation of pesticides were most prominent.

A long duration of exposure may reflect a different exposure situation compared with a short duration of exposure. As different determinants of exposure prevailed for both subgroups, it should be considered to construct exposure models for both groups separately.

Dislodgeable foliar residue (DFR) was the most prominent determinant of exposure for both respiratory and dermal exposure during re-entry. However, no

[1] Johan de Cock, Dick Heederik, Hans Kromhout, Jan S.M. Boleij, Fred Hoek, Hillion Wegh, Evelyn Tjoe Ny, Submitted for publication

significant relation between DFR and dermal exposure of forehead and sternal area was found. This may be explained by indirect exposure of these skin areas as compared with direct contact of other body parts such as hands and arms with foliage. Therefore, it was concluded that use of a transfer factor based on dislodgeable foliar residue to estimate total dermal exposure is only a crude estimate.

The half-life of captan on crops varied from 10 - 17 days, and so substantial exposure when entering the orchard is very likely to occur, particularly when spraying frequency is high.

The main starting points for reduction of exposure are use of a cabin, dislodgeable foliar residue and individual time spent on different tasks. Especially determinants which are constant over time (cabin use) may have a great influence on grouping workers according to long term exposure in epidemiological studies.

As determinants of exposure vary for the different exposure routes and body locations (for dermal exposure), the measure of interest for a specific study design will decide which determinants are most relevant.

INTRODUCTION

The study of occupational exposure to pesticides is complex, as it concerns exposure to several compounds, each with different contributions to dermal and respiratory uptake. A series of studies were carried out on occupational exposure to pesticides among fruit growers in The Netherlands during spraying and re-entry of the crops between 1990 and 1992 to quantify and identify determinants of exposure. Captan was used as a marker of exposure, which is the most commonly used fungicide in The Netherlands. Its use of over 300.000 kg in 1992 was 17 times higher than the second most commonly used fungicide on hard fruit (CBS, 1992).

Differences between tasks in type of exposure, may lead to differences in health risk. For instance, preparation of a tank mixture concerns peak levels of exposure to non-diluted chemicals over a short duration. In comparison, exposure in the orchards, during re-entry (*i.e.* tasks performed in the orchards other than application) concerns a more constant, lower level of exposure to a mixture of pesticide residues over a longer time.

Good hygienic practice will aim at reducing exposure as far as possible. Therefore, data on determinants of exposure can form the basis for measures to be taken to achieve this goal for large groups when it is impossible to gather measurement data on individual level. Popendorf and Leffingwell (1982) and Nigg *et al.* (1984) proposed for example an empirical factor for the estimation of dermal exposure to pesticides by field workers based on the relationship between the dermal exposure rate (mg hr^{-1}) and dislodgeable foliar residue mg cm^{-2} (DFR). Data on determinants can be used for exposure assessment in epidemiological studies as well. An accompanying paper in this issue focuses on the contribution of exposure route, performed tasks, season and environment to total exposure, with special attention to variability in exposure (de Cock *et al.*, 1995).

In this paper, determinants of exposure will be discussed as a starting point for hazard identification and control *i.e.* measures to be taken to reduce occupational exposure to pesticides.

MATERIALS AND METHODS

The study population, measurement strategy, and analytical procedures are described in Chapter 2.

Statistical Analysis

All data analyses were performed using Statistical Analysis System Software (SAS). The exposure distribution was tested for (log)normality with PROC UNIVARIATE. PROC TTEST and PROC NPAR1WAY MEDIAN were used to compare average exposure of subgroups. PROC REG was used to study the relation between log-transformed exposure variables and determinants of exposure in univariate and multivariate analyses. Univariate linear regression analyses of log normally transformed exposure variables were used to select determinants of exposure which explained at least

10% of the variability in exposure and had a *P*-value less or equal 0.05. Also multiple regression analyses were performed. Stepwise regression analysis was used and a significance level of 0.50 was used for entry in the model. A variable was kept in the model at a significance level of 0.10.

RESULTS

Application

Determinants considered based on preliminary univariate analyses were: *use of a cabin, type of airblast sprayer, mower behind the tractor, use of a cabin filter, tank content, number of sprayings, type of spraying nozzles, type of fan on the sprayer, location of preparation of a tank mixture, filling directly from package into the tank, use of rinsing equipment, amount of active captan sprayed, amount of diluted captan sprayed, duration of mixing and loading, duration of spraying, fruit growers opinion about own exposure, use of gloves, respiratory protection, age of fruit grower, and use of head covering.*

As use of a cabin appeared to have a predominant effect, results of regression models are presented for both groups separately in Table 1 and 2. The models explained 40-60% of the variability in respiratory exposure, but only 16-50% of variability in dermal exposure.

Respiratory exposure was determined by factors related to preparation of tank mixtures. Time spent on mixing and loading was a determinant related to respiratory exposure for both cabin and non-cabin users. For *non-cabin users*, a mower between the tractor and the sprayer was related to lower respiratory exposure. Use of gloves and a larger amount of captan sprayed was related to a higher respiratory exposure in this group. For *cabin users*, mixing and loading partly inside a shed, led to higher respiratory exposure. Actual use of a (coal and/or dust) filter and adding the pesticide preparation directly into the tank was related to lower exposure. The age of the fruit grower was a determinant of exposure for cabin-users, showing a higher exposure for older fruit growers. For cabin-users, the opinion of fruit growers on their exposure as compared with a 'normal' situation and use of respiratory protection were associated with higher exposure.

Table 1 Multivariate linear regression analysis of exposure characteristics related to log-transformed exposure variables during mixing/loading and application of captan without a cabin, fruit growing, The Netherlands, 1990

No Cabin	%[A]	Mean [range]	Regression Coefficient	Standard Error	*P*-value	R^2 [B]
Inhalable dust (n = 45) ($\mu g\ m^{-3}$)						0.40
Intercept			1.67	0.47	< 0.01	
Duration mixing/loading (min)		11 [0-33]	0.07	0.02	< 0.01	
Mower (1/0)	28%		- 0.84	0.39	0.04	
Gloves, mixing and/or spraying (1/0)	33%		0.81	0.38	0.04	
Amount active captan sprayed (kg)		3.7 [0.3-10]	0.19	0.09	0.04	
Wrists (n = 45) ($mg\ m^{-2}\ hr^{-1}$)						0.28
Intercept			0.59	0.33	0.08	
Direct from package in tank (1/0)	28%		1.0	0.36	0.01	
More than one spraying completed (1/0)	15%		0.83	0.45	0.07	
Higher exposed than normally (1/0)	71%		0.95	0.35	0.01	
Arms (n = 43) ($mg\ m^{-2}\ hr^{-1}$)						0.25
Intercept			- 0.39	1.19	0.75	
Amount diluted captan sprayed (l)		742 [50-4000]	$0.73\ 10^{-3}$	$0.42\ 10^{-3}$	0.09	
Tank content (l)		850 [500-1200]	$2.35\ 10^{-3}$	$1.37\ 10^{-3}$	0.09	
Respiratory protection, spraying (1/0)	22%		1.48	0.59	0.02	
Neck (Back) (n = 39) ($mg\ m^{-2}\ hr^{-1}$)						0.16
Intercept			-0.45	0.42	0.29	
Direct from package in tank (1/0)	28%		0.80	0.42	0.07	
Higher exposed than normally (1/0)	71%		0.95	0.44	0.04	
Forehead (n = 37) ($mg\ m^{-2}\ hr^{-1}$)						0.29
Intercept			1.01	0.47	0.04	
Gloves during mixing and loading (1/0)	33%		- 1.73	0.52	< 0.01	
Higher exposed than normally (1/0)	71%		1.06	0.54	0.06	

[A] % = percentage of farmers with characteristic; [B] R^2 = explained variance.

Table 2 Multivariate linear regression analysis of exposure characteristics related to log-transformed exposure variables during mixing/loading and application of captan with a cabin, The Netherlands, 1990

Cabin	%[A]	Mean [range]	Regression Coefficient	Standard Error	*P*-value	R^2 [B]
Inhalable dust (n = 46) ($\mu g\ m^{-3}$)						0.61
Intercept			- 1.8	1.0	0.08	
Mixing/loading inside (1/0)	35%		1.1	0.42	0.01	
Duration mixing/loading (min)		16 [0-59]	0.08	0.02	< 0.01	
Direct from package in tank (1/0)	37%		- 1.13	0.43	0.01	
Opinion: exposure higher than normally (1/0)	71%		1.16	0.44	0.01	
Age of fruit grower (y)		47 [24-68]	0.07	0.02	< 0.01	
Actual use filter on cabin (1/0)	50%		- 1.15	0.40	0.01	
Respiratory protection, mixing/spray (1/0)	35%		1.01	0.42	0.02	
Wrists (n = 52) ($mg\ m^{-2}\ hr^{-1}$)						0.17
Intercept			1.76	0.37	< 0.01	
Mower (1/0)	37%		- 1.32	0.45	0.01	
Conventional sprayer (1/0)	50%		- 0.84	0.44	0.06	
Arms (n = 42) ($mg\ m^{-2}\ hr^{-1}$)						0.50
Intercept			- 4.31	0.82	< 0.01	
Amount diluted captan sprayed (l)		1015 [100-4075]	$0.71\ 10^{-3}$	$0.29\ 10^{-3}$	0.02	
Age of fruit grower (y)		47 [24-68]	0.07	$0.01\ 10^{-3}$	< 0.01	
Propeller Fan (1/0)	60%		1.19	0.34	< 0.01	
Respiratory protection, mixing/loading (1/0)	33%		- 0.72	0.36	0.06	
Neck (Back) (n = 32) ($mg\ m^{-2}\ hr^{-1}$)						0.21
Intercept			- 0.97	0.22	< 0.01	
Mower (1/0)	37%		- 0.60	0.31	0.06	
Respiratory protection, mixing/loading (1/0)	33%		0.68	0.32	0.04	
Forehead (n = 28) ($mg\ m^{-2}\ hr^{-1}$)						0.45
Intercept			- 1.70	0.39	< 0.01	
Duration mixing/loading (min)		16 [0-59]	- 0.03	0.02	0.07	
Actual use filter on cabin (1/0)	50%		0.53	0.30	0.09	
Propeller Fan (1/0)	60%		1.09	0.31	< 0.01	

[A] % = percentage of farmers with characteristic; [B] R^2 = explained variance.

Dermal exposure of *wrists* of fruit growers without a cabin was related to work style during preparation of tank mixtures (adding captan directly from package into tank), total number of sprayings, and fruit growers' personal opinion on their exposure. For cabin-users, wrist exposure was determined by type of sprayer and the presence of a mower behind the tractor, but explained variance was low (17%).

Exposure of the *arm* for non-cabin users was positively associated with the amount of captan solution sprayed, the tank content and those using respiratory protection. For fruit growers with a cabin, a propeller fan on the sprayer, amount sprayed, and age of fruit growers were associated with higher arm exposure. Wearing of respiratory protection during preparation of tank mixtures was associated with lower exposure for this group of cabin users.

For the *back of the neck*, for non-cabin users, a tank mixture prepared directly from the package into the tank and fruit growers' opinion on exposure were associated with higher exposure. For cabin-use exposure was positively associated with wearing of respiratory protection during preparation of tank mixtures and negatively associated with use of a mower during spraying. Explained variance, however, was low for dermal exposure for both cabin-users (21%) and non-cabin users (16%).

For the *forehead*, for non-cabin users, gloves during mixing and loading was associated with lower exposure of forehead and fruit growers' personal opinion was related to higher exposure of the forehead. For cabin users a short duration of preparation of tank mixtures, actual use of a cabin filter and a propeller fan contributed to higher exposure. Explained variance was relatively high (45%).

Exposure duration

As a long duration of exposure may reflect a different exposure situation compared with a short duration of exposure, this may influence the relationship between exposure variables and determinants under study. Therefore, models were also calculated for observations with a measurement duration of at least two hours, for the same set of independent variables. Generally, explained variance was higher compared with models including all observations. As expected, most independent variables also contributed significantly for this subset of observations. A stepwise multiple regression analysis, however, revealed a different selection of independent variables compared with the models based on all observations.

For dermal exposure of wrists, arm and neck generally the same determinants appeared to be relevant. For dermal exposure of forehead, age of fruit growers, use of a propeller fan on the sprayer and actual use of rinsing equipment, replaced the use of gloves and respiratory protection.

For respiratory exposure a different model was described. A stepwise regression model was used for measurement shorter than two hours and a model for two hours or longer. For both models different determinants of exposure seemed relevant. For the former group, use of a cabin, duration of preparation of a tank mixture, number of performed sprayings, actual use of a cabin filter, total crop area, amount of active compound sprayed and wearing of respiratory protection during mixing and loading were determinants of respiratory exposure. In the latter group determinants were number of tank loadings prepared, adding captan directly into the tank, amount of diluted captan sprayed, wearing of gloves during spraying, age of farmer, and use of swirl nozzles on the sprayer. Both, models described respiratory exposure better for the subgroups in question than a general model based on all observation together (R^2 = 37%, N = 91; data not shown). Only the variables, 'adding captan directly from the package into the tank' and 'age of the fruit grower' contributed significantly in both models.

Preparation

Exposure during preparation of a tank mixture was measured exclusively in a small subset (n = 23). Dermal exposure of wrists (AM = 71.0 mg m^{-2} hr^{-1}, range: 0.75-837) was negatively related to duration of preparation (min), (regression coefficient -0.15, SE 0.05, R^2 0.32, $P<0.01$). Exposure of wrists was positively associated with total crop area, and a negative association was found for actual use of a cabin filter and use of a conventional type of sprayer.

Re-entry

Determinants considered based on preliminary univariate analyses were: *dislodgeable foliar residue (DFR), foliar density of crop, planting system, captan dose* *applied, number of days since last spraying, experience of subject with performed tasks, length, weight, and age of subject, type of sprayer, sprayed area, used personal protection of head and face, wearing of coat, trousers, and wearing of rubber boots.*

Dislodgeable foliar residue (DFR) was the most prominent determinant of respiratory and dermal exposure during re-entry. The regression coefficient of exposure on DFR and explained variance was higher for data collected in 1991 compared with 1992-data. DFR of captan on fruit farms did not differ significantly between both years (Table 3), but number of days elapsed since the last spraying was higher in 1991 than in 1992 (1991: 1-43 days; 1992: 0-21 days). The range in the amount of captan applied was somewhat smaller in 1991 (1991: 249-1294 g/ha; 1992: 208-1971 g/ha).

Multiple regression analyses revealed that exposure could be explained by dislodgeable foliar residue and year of exposure for respiratory exposure and dermal exposure of wrists, arm and forehead (Table 4). Exposure in 1992 was lower. For hands, year of exposure did not contribute significantly. Besides DFR and year of exposure, contribution of other variables was small and explained less than 5% of variance (data not shown).

Decay of captan

Half-life of captan on leaf surfaces is presented in Table 5, assuming a first order decay. For 1991, data were based on dislodgeable foliar residue (DFR) on one lot followed during 30 days after application with captan. Amount of captan sprayed was 83 mg m^{-2} (figure 1). Over a longer period of 99 days, 4 sprayings were performed on the same lot. As initial applied amount of captan varied for different sprayings (83-249 mg m^{-2}), half-life time was calculated using the amount relative to the initially quantity of captan applied. For the 1992-data a similar approach was used, but data originated here from 19 different farms. The estimated half-life time of captan varied between 10 and 17 days. Explained variance was highest (69%) when half-life was calculated using data from one farm and lowest (33%) when based on different farms and different amounts sprayed.

Table 3 Dislodgeable foliar residue (DFR) for two sided leaf area (mg m^{-2}) on fruit growing farms in The Netherlands

Year	N	AM	GM	GSD	Range
1991	29	19.0	15.2	2.1	2.7 - 46
1992	40	21.1	13.9	2.8	0.26 - 131

Table 4 Multivariate linear regression analysis of exposure characteristics related to log-transformed exposure variables; re-entry of orchards treated with captan, on log-transformed foliar dislodgeable residue, fruit growing, The Netherlands, 1991 and 1992

Re-entry 1991 and 1992	Regression Coefficient	Standard Error	*P*-value	$R^{2\,B}$
Inhalable concentration (n = 149)				0.37
Intercept	0.36	0.42	0.39	
DFR[A]	0.84	0.11	< 0.01	
Year (1992/1991, 1/0)	- 1.14	0.21	< 0.01	
Hands (n = 177)				0.58
Intercept	- 1.72	0.25	< 0.01	
DFR	1.10	0.07	< 0.01	
Year (1992/1991, 1/0)	0.16	0.14	0.26	
Wrists (n = 183)				0.49
Intercept	- 2.63	0.28	< 0.01	
DFR	0.98	0.08	< 0.01	
Year (1992/1991, 1/0)	- 0.73	0.16	< 0.01	
Arm (n = 171)				0.37
Intercept	- 2.62	0.32	< 0.01	
DFR	0.86	0.09	< 0.01	
Year (1992/1991, 1/0)	- 0.54	0.17	< 0.01	
Forehead (n = 176)				0.17
Intercept	- 3.92	0.38	< 0.01	
DFR	0.60	0.11	< 0.01	
Year (1992/1991, 1/0)	- 0.39	0.21	0.06	

[A] Dislodgeable Foliar Residue (mg m^{-2}); [B] R^2 = explained variance.

Table 5 Decay of captan on leaf surface, first-order kinetics

Year	N	Explained variance	Half-life (days)	95%-CI (days)
1991 (one farm, one spraying)	15	0.69	17	12 - 27
1991 (one farm, 4 sprayings)	28	0.50	13	5 - 21
1992 (19 farms, 30 observations)	30	0.33	10	7 - 22

95%-CI = 95 percent confidence interval.

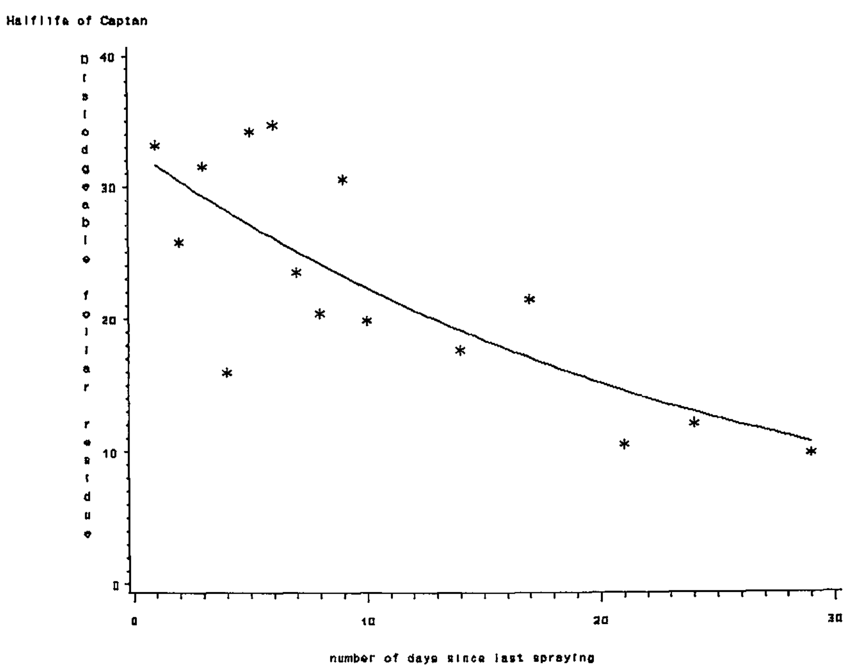

Figure 1 Decay of DFR on one lot sprayed with captan, fruit growing, 1991

Table 6 Multivariate linear regression analysis between dermal exposure (log-transformed) and log-transformed independent variables inhalable dust concentration and dislodgeable foliar residue; re-entry of orchards treated with captan, The Netherlands, 1992

Re-entry 1992	Regression Coefficient	Standard Error	*P*-value	$R^{2\,C}$
Hands (n = 62)				0.39
Intercept	- 3.80	0.49	< 0.01	
DFR[A]	0.65	0.15	< 0.01	
PAS[B]	0.20	0.08	0.02	
Wrists (n = 60)				0.56
Intercept	- 2.72	0.49	< 0.01	
DFR	0.48	0.15	< 0.01	
PAS	0.49	0.08	< 0.01	
Arm (n = 56)				0.49
Intercept	- 2.62	0.55	< 0.01	
DFR	0.43	0.17	0.02	
PAS	0.47	0.09	< 0.01	
Forehead (n = 56)				0.14
Intercept	- 2.76	0.57	< 0.01	
DFR	- 0.046	0.18	0.80	
PAS	0.29	0.09	< 0.01	
Sternal (n = 57)				0.00
Intercept	- 3.05	0.35	< 0.01	
DFR	0.07	0.11	0.51	
PAS	0.05	0.06	0.43	

[A] Dislodgeable foliar residue (mg m^{-2}): n = 65, mean = 45.5, range: 0.53-262; [B] Personal air sampling (PAS) of inhalable dust ($\mu g\ m^{-3}$): n = 63, mean = 23.6, range: 0.17-256; [C] R^2 = explained variance.

Dislodgeable foliar residue

Personal inhalable dust concentration was related to dislodgeable foliar residue (DFR). For DFR a significant positive relation was found with dermal exposure of hands, wrists and arm, but not with forehead and sternal area. Except for sternal area, dermal exposure also was related to inhalable dust concentration. In Table 6 it is shown that for hands, wrists and arm, both DFR and inhalable dust concentration contributed to dermal exposure.

DISCUSSION AND CONCLUSIONS

For epidemiological study purposes, exposure is estimated for instance over the period of one season, a total year or several years. In this perspective determinants of exposure can be divided into the following categories: 1) determinants which are 'fixed' such as crop area, farm type, use of a cabin, which may be constant over time; 2) determinants, which vary from day to day but which may not be influenced by the subject such as dislodgeable foliar residue; 3) determinants which are variable and may be influenced by a subject such as wearing of personal protection equipment, work style and hygienic behaviour; and 4) determinants which may not be influenced such as weather conditions. Especially, fixed determinants, which are constant over time, with a strong influence on exposure have a great influence on grouping workers according to long term exposure. Such determinants, however, may only be valid for one specific exposure measure. For instance, cabin use during spraying had a strong influence on dermal exposure, for example a 12-fold reduction of exposure for the forehead, while exposure of hands and respiratory exposure was similar for cabin users compared with non-cabin users. During re-entry, however, the opposite came apparent. Exposure of forehead was not explained by DFR, whilst exposure of the hands was.

Application

A cabin had most prominent influence on dermal exposure. Respiratory exposure, however, was explained predominantly by factors related to mixing and loading of undiluted captan dust. Interpretation of results from subgroups with and without a cabin on the tractor is complicated by other, underlying, determinants of exposure. Beside a direct protecting effect, use of a cabin also may be related to different hygienic behaviour in general or may reflect differences in other farm characteristics. A striking finding in this respect was that cabin use was related to lower dermal exposure, though farms where farmers used a cabin had larger median crop area (12 *vs* 9 ha, Chi square: P=0.01), and more captan was sprayed (5 kg *vs* 3.3, Chi square: P=0.07). Also duration of preparation of a tank mixture (13.5 *vs* 9 min; Chi square: P=0.02) and total duration of application (125 *vs* 97 min, Chi square: P<0.01) was longer for cabin users. Additionally, median tank content was larger (1000 *vs* 800 litre; Chi square: P<0.01) which reflects more modern farm practices of cabin users compared with more use of a conventional type of sprayer among non-cabin users (Chi square: P=0.03). For respiratory exposure, explained variance was higher than explained variance for dermal exposure. This may be due to differences in variability within and between workers for both exposure routes. Work-style, personal hygiene and behaviour will lead to larger differences between workers, but were not considered in this study.

For fruit growers spraying without a cabin, use of gloves is associated with higher respiratory exposure, which may be due to differences in work style. Fruit growers may feel safe when using gloves, or gloves are used because a cabin on the tractor is absent. Fruit growers' personal opinion should be regarded as an explanatory variable rather than a determinant of exposure. This variable could indirectly reflect other determinants of exposure. On the other hand, this variable is likely to explain unmeasured determinants such as unusual weather conditions or sloppiness during work, which were frequently given motivations on their opinion about their exposure. This variable was kept in the models, since it seemed to explain exposure independently of the other variables.

For fruit growers who used a cabin while spraying, the model for respiratory exposure was more pronounced. Fruit growers' personal opinion also explained differences in exposure between farmers. It is uncertain, if the use of a filter in the cabin really reduced respiratory exposure or reflects personal behaviour of this group, indirectly resulting in lower exposure. The age of the fruit grower possibly reflects underlying factors such as differences in equipment used, work style and hygienic behaviour.

A positive association found for dermal exposure of the back of the neck and wearing of respiratory protection during preparation of tank mixtures was probably caused by putting on contaminated equipment or putting of equipment with contaminated hands. This was also found for exposure of forehead during mixing and loading (N = 10, regression coefficient 3.15, SE 0.57, R^2 0.80, *P*-value <0.01). It is not clear why a mower is associated with lower exposure for fruit growers with a cabin, unless the cabin was not closed during spraying. In that case the greater distance between tractor an sprayer may explain a lower exposure. Dermal exposure of forehead was higher when gloves were not used during mixing and loading, which is possibly due to secondary contamination when fruit growers touch their skin with contaminated hands after mixing and loading.

Duration of activities

Short duration of measurement may be related to the relationship between exposure and its determinants. Duration typically is low for the application of pesticides as compared with normal 8-hour workshifts. Differences, varying from less than 30 minutes to almost 5 hours, may be due to circumstances on that typical day as well as to more general farm characteristics. Farms with a measurement duration less than two hours had a smaller total crop area (9.9 hectare) than other farms (12.2 hectare; *t* test, $P<0.03$). This also resulted in significant lower amounts of active compound sprayed (3.4 and 6.8 kg, respectively). Short measurement duration seems to reflect a typical situation for a specific group of fruit farms and those observations should not be excluded only for the reason of short measurement duration. As, different determinants of exposure may prevail for both subgroups, it should be considered to construct exposure models for both groups separately.

In general, cabin use was by far the most important determinant of dermal exposure. Some other determinants may contribute to some extent as was shown for subgroups according to cabin use. No uniform model for the different dermal exposure locations was observed.

Re-entry

Dislodgeable foliar residue (DFR) was the most prominent determinant for respiratory and dermal exposure during re-entry. Differences between 1991 and 1992 seem to be due to differences in tasks performed and time elapsed since last spraying. In 1991 harvesting was the main activity observed (83%). This task may lead to a more intensive contact with crop and leaves than other tasks such as pruning, which may have led to the more profound relationship observed with DFR. Explained variance of other variables typically was low in comparison with DFR.

One-row planting system contributed to lower respiratory, and lower dermal exposure of hands and wrists in 1991 and of forehead in 1992. A one-row planting system most likely leads to less contact with crop. Subjects' age and experience with performed tasks may have led to differences in working methods, resulting in the observed relationship with higher dermal exposure of hands and arm in 1991 as compared with 1992. A positive relation between exposure of wrists with number of days since last spraying in 1991 may be due to some farms with relatively high DFR after a long duration of time.

For exposure of the hands based on hand rinse-data, year of exposure did not contribute significantly to explained variance in multivariate analyses. A stronger relation for hands based on hand rinsing compared with wrist-data may be explained by differences in skin location. Total hand exposure based on hand rinsing reflecting contact with foliage better than dermal exposure based on wrist pads.

The estimated half-life of captan varied from 10 - 17 days (95% CI: 5 - 27 days), indicating that substantial exposure when re-entering the lots is likely to occur. Although differences in DFR may depend on climate and crop type, the results were in the same order of magnitude as described in literature (6 - 15 days). Most of the studies concerned decay on strawberries (Ritcey *et al.*, 1987; Stamper *et al.*, 1987; Winterlin *et al.*, 1984; Zweig *et al.*, 1985) and on grapes (Winterlin *et al.*, 1986). When the frequency of application is high, even accumulation is likely to occur in some periods of the spraying season. Additionally, lots which have been sprayed with a high dose, still may have high DFR levels even after a few weeks.

Transfer factor for DFR

Zweig (1985) presented a crude approximation for dermal exposure rate for fruit harvesting as dermal exposure rate = 5000 (cm^2 hr^{-1}) × DFR (for a single projected leaf surface). The units of the ratio represent the foliar surface area of contact. During re-entry tasks in fruit growing, the transfer factor between the dermal exposure rate (1.28 mg hr^{-1}), which was based on hand rinsing (2 × 360 cm^2) and exposure of the arm (2 × 600 cm^2), and dislodgeable foliar residue (45.5 mg m^{-2}) was much lower (mean: 346 cm^2 hr^{-1}, SD 278, N = 65). Generally, the observed DFR was in the same order of magnitude as DFR for Chlorothalonil, Thiophanate-methyl, Thiram and Zineb varying from 12.1 - 50 mg m^{-2} as found by Brouwer *et al.* (1992) in the cultivation of carnations in greenhouses. Dermal exposure in our study was lower, although amounts on leaves were comparable. Observed differences may be caused by differences in used measurement methods, dimension of skin pads, projected skin area for calculating dermal exposure or differences in activity pattern. Fenske (1989) showed that fruit picking rate influenced exposure. It is likely that dermal exposure is a function of both active or direct contact with contaminated surfaces, and a function of passive deposition of particles available in the direct environment (air) of workers, caused by the activities performed and intensity of contact with the foliage. No significant relation between DFR and dermal exposure of forehead and sternal area was found in our study which may be explained by indirect exposure of these skin areas as compared with direct contamination by contact of hands and arms with foliage. Use of a transfer factor based on DFR to estimate total dermal exposure, therefore can only be a crude estimate of exposure. It should be noted here that inhalable dust concentration and DFR were mutually related (N = 67 regression coefficient 0.85, SE 0.18, R^2 0.26, *P*-value <0.01). Dependency between both variables, however, is complicated by activity levels of the worker and differences between dislodgeable and available foliar residue, which may also be influenced by external factors as temperature and humidity during exposure. Also, inhalable dust concentration does not take into account larger airborne particles, which may also contribute to dermal exposure.

As the population of fruit growers can be grouped based on external exposure, the determinants which explain exposure can be used for control strategies to reduce exposure. Based on the results the main starting points would be cabin use, dislodgeable foliar residue and individual time spent on different tasks. Whether exposure data can also be used for compliance with standards depends on how exposure limits for dermal exposure to pesticides will be set in future. To set limit values based on the internal dose received via multiple route exposure seems impractical, since this will need a biomonitoring method for each pesticide or group of pesticides.

It was suggested that it seems feasible considering the development of dermal occupational exposure limits (DOEL) for selected workplaces and environmental agents (Fenske, 1993). These DOEL's are based on surface sampling. As we found only a clear relationship with DFR for some skin areas and also a contribution of the airborne concentration on dermal exposure, it could be considered if an indirect effect of airborne concentration on dermal exposure should be taken into account. It was recently shown that a strategy may be agent dependent, since for exposure to captan only the specific skin areas of neck and ankles contributed to uptake (de Cock *et al.*, 1995b).

On balance, as determinants of exposure vary for the different exposure routes and body locations (dermal exposure), the measure of interest for a specific study design will decide which determinants are most relevant in a study (exposure measurements, use of questionnaires), the type of health effects (acute, chronic) or type of biological monitoring (blood, urine). It is obvious that occupational exposure limits only based on the respiratory route should be considered out of date, but it is doubtful if a skin notation for pesticides with a high skin permeability will be sufficient.

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CHAPTER 4

Subjective assessment of pesticide exposure in fruit growing by experts[1]

ABSTRACT

Exposure to pesticides in fruit growing was estimated by experts with different professional expertise, respectively pesticide experts, occupational hygienists and fruit growing experts to see whether valid subjective assessments can be made by experts. The study objectives were: 1) validation of exposure assessment by experts using different sources of information; 2) assessment of inter-rater agreement; 3) measurement of agreement between experts' assessment and actual quantitative exposure data.

An expert panel of three groups with different expertise, made four ratings. For three of these ratings assessments were made in three phases in each of which exposure information was provided.

Intra-class correlation was high for each subgroup of experts when tasks in fruit growing were relatively ranked by increasing exposure level. In general, inter-rater agreement on factors influencing the internal dose decreased when more information on exposure was provided. All experts correctly considered dermal exposure as the prominent contributor to internal dose. Results were comparable for three pesticides under study. Ranking of actual exposure situations of 15 specific sprayings with a fungicide,

[1] Johan de Cock, Hans Kromhout, Dick Heederik, Jan Burema, Submitted for publication

clearly showed differences between raters according to their expertise. The pesticide experts and occupational hygienists were able to rank daily exposure levels during pesticide spraying in a meaningful way.

The experts seem to recognize the most important determinants of external exposure and therefore may play a role in evaluating the effectiveness of control measures taken to reduce external exposure. Preferably, an expert panel should not be too small, and consensus or average estimates should be used, because differences within expert groups can be considerable.

INTRODUCTION

Exposure to pesticides in agriculture is complex, considering that a variety of factors may influence exposure and uptake into the body. Over the last decade, growing attention has been given to the skin as route for uptake. Data on skin exposure are difficult to interpret, because of lack of information on skin absorption under working conditions and therefore lack of information on its contribution relative to inhalation and ingestion.

Epidemiological studies often involve effects of long term exposures, which occurred long before the manifestation of disease. This may result in problems because of the absence of adequate monitoring in the past (Goldberg, 1993). Job titles and job exposure matrices, often developed for a specific industry, have been used to distinguish occupationally exposed workers in epidemiological studies. Since occupational exposure to pesticides is complex because of the variety of compounds being used, a crop exposure matrix (CEM) was proposed (Miligi *et al.*, 1993). The CEM relates agricultural practices to pesticide exposures taking into account changes over time and the use of chemicals by farm area. Subjective assessments by experts to characterize occupational exposure have so far been used mainly in industry (Kromhout *et al.*, 1987; Hertzman *et al.*, 1988; Hawkins *et al.*, 1989; Teschke *et al.*, 1989; Post *et al.*, 1991).

To see whether experts can estimate a complex situation of outdoor exposure in agriculture, pesticide exposure of fruit growers in The Netherlands has been estimated by

15 experts. As part of the EC concerted action "Retrospective evaluation of occupational exposures in cancer epidemiology" (Hémon, 1993), cooperative studies were carried out in agriculture (vineyards), shoe and leather making and metal plating in Italy (Segnan *et al.*, 1995) and fruit growing in The Netherlands. This study has been carried out in close cooperation with the vineyard study of the Epidemiology Unit, Department of Oncology, in Torino, Italy.

The study objectives were: 1) validation of exposure assessment by experts using different sources of information; 2) assessment of inter-rater agreement; 3) measurement of agreement between experts' assessment and actual measured exposure data.

STUDY DESIGN AND METHODS

Experts

A panel of 15 experts was asked to subjectively assess exposure. The panel consisted of general occupational hygienists, occupational hygienists working at research institutes with experience in the field of pesticide exposure, and experts on fruit growing. Each group consisted of five raters. The three groups will be referred to as respectively occupational hygienists, pesticide experts, and fruit growing experts.

Information phases

Assessments were made after three consecutive information phases. During each of these phases information on fruit growing and pesticide exposure was presented. The assessments took place during a one day meeting in November 1992. This approach was chosen, because work in fruit growing concerns seasonal activities, most of which are weather dependant, so that inspection of the different work situations would not have been possible by visiting fruit farms. After each information phase experts were asked to make separate assessments for three different pesticides.

Ratings

In each phase the following ratings were made:

(A) A ranking of external dermal and respiratory exposure according to performed tasks. Raters were asked to rank 14 tasks in fruit growing for external dermal and respiratory exposure according to performed tasks, attaching rank number one to the lowest exposure (Table 1).

(B) An estimation of the influence of different factors on the internal exposure dose. Factors (14) to be assessed concerned the influence of type of (spraying)equipment, personal protection equipment and weather conditions (Table 2). The influence was categorized as follows: "strongly reducing (--)", "reducing (-)", "no influence or irrelevant", "increasing (+)" or "strongly increasing (++)". The assessments had to be made for the application of pesticides and re-entry (tasks performed in the orchards other than application) separately. Inter-rater agreement was expressed as Cohen's Kappa. Kappa statistic has an interpretation as intra-class correlation coefficient (ICC) (Ebel, 1951). For the case of multiple ratings per subject, the mean Kappa was calculated as weighted average as proposed by Landis and Koch in 1977, cited by Fleiss (1981). In case of equal numbers of ratings per factor, approximate standard errors for Kappa were calculated for testing the hypothesis that the underlying value is zero as proposed by Fleiss, Nee and Landis in 1979, cited by Fleiss (1981). The ICC is a widely used measure of inter-rater reliability for the case of quantitative ratings. The five aforementioned categories were reduced to three by taking (-- and -) together and also (+ and ++).

(C) Estimation of the relative contribution of exposure route on the dose. Raters assessed the relative contribution of exposure route to the total dose by giving a percentage for the dermal, respiratory and the oral contribution respectively. Differences were studied using nonparametric statistics (Wilcoxon χ^2).

(D) A random sample of 15 farms was taken from an exposure database of 57 farms, concerning application of a fungicide (captan) with an airblast sprayer (Table 3). A description was given of the main farm and task characteristics, without presenting actual exposure data (Table 4). Characteristics of individual captan sprayings on 15 different fruit growing farms, included information on crop type, mixing and loading, type of equipment, personal protection equipment, and weather conditions. A single time experts were asked to rank the farms for external dermal and respiratory exposure and the internal exposure dose, attaching rank number one to the lowest exposure. Analysis of variance was used to calculate the intra-class correlation (Annex 1). The ranking was validated by calculating correlation coefficients between the ideal (measured) ranking compared to the ranking made by the experts. Also actual measured exposure was compared between farms with rank number 8 or higher and rank numbers 1-7 by a two-tailed *t* test to study if ranking resulted in groups of farms with different exposure levels.

Table 1 Tasks in fruit growing (Method A)

Number	Task
1.	mixing/loading liquid pesticide from 1-litre package
2.	mixing/loading powdery pesticide from 25-kg package
3.	spraying with spray-gun (tank content 10 litre)
4.	spraying with airblast sprayer without a cabin
5.	spraying with airblast sprayer with a cabin
6.	tasks performed inside a shed
7.	tasks in crop: pruning
8.	tasks in crop: tying up
9.	tasks in crop: bending
10.	tasks in crop: thinning
11.	harvesting
12.	transport of fruit from orchard to store
13.	sorting of fruit by hand
14.	planting and treatment against fruit tree canker.

Table 2 Factors, potentially influencing exposure (Method B)

Number	Factor
1.	cabin on tractor
2.	high concentration in tank
3.	large tank content
4.	gloves
5.	dust mask
6.	respirator (dust/active coal filter)
7.	spraying suit
8.	boots
9.	head wind
10.	side wind
11.	wind behind
12.	high wind speed
13.	high temperature
14.	high humidity.

Table 3 Descriptive statistics of a random sample of 15 farms from a database of 57 captan-sprayings (Method D)

Exposure route	n	Arithmetic Mean	Standard Deviation	Range
respiratory ($\mu g\ m^{-3}$)	15	61.7	69.2	1-202
dermal, *total* (mg $hour^{-1}$)	9[A]	12.4	6.9	3.5-21.0
dermal, *wrists* (mg m^{-2} $hour^{-1}$)	15	9.0	7.6	0.4-25.3

[A] Due to missing values for some skin locations, not for all sprayings total dermal exposure was calculated.

Table 4 Example of farm and task characteristics

Farm number 1
location: Flevopolder
crop area hard fruit: 8 hectare

Spraying with captan

age of fruit grower .... (years) 63

DATA ON SPRAYED PARCELS
number of sprayed parcels 1
number of trees per hectare 2000
average height of trees .... (cm) 200
average age of trees .... (year) 3 (2 - 4)
planting system (number of rows) single
direction of paths between trees .. north-south
wind direction on the farm .... north

WEATHER CONDITION (station Wageningen)
date 21AUG90
mean day temperature ...... (Celsius) 14.1
relative humidity .... (%) 85

MIXING/LOADING
time work started 10:38
duration of task .... (min) 5
mixing/loading inside? .... yes
door open during task? .... open
directly from package in tank? .... yes
number of packages of captan used 1
rinsing installation used? .... no

SPRAYING
duration of task .... (min) 124
type of sprayer .... conventional
type of fan on the sprayer ...... centrifugal
cabin on tractor? .... no
mower between tractor and sprayer? ..... no
maximal tank content of sprayer .. (litre) 800
spraying type: misting? .... yes
swirl nozzle .... no
amount sprayed active captan ..... (kg) 3.32
tank concentration captan .... (g/l) 7.38

CLOTHING WORN DURING WORK
shirt .... yes
sweater .... yes
trousers .... yes
- shorts .... no
- trousers .... yes
- rainproof trousers .... yes
coat .... yes
raincoat .... yes
gloves .... no
- shoes .... no
- wooden shoes/clogs .... yes
- boots .... no
hat/cap .... yes
face protection (glasses) .... no
respirators .... no
hearing protection .... yes

OPINION OF FRUIT GROWER
ABOUT EXPOSURE: ...... normal situation

Exposure was defined as contact during mixing/loading and/or spraying or during re-entry or other work activities on the farm. External exposure was defined as potential exposure, *i.e.* the amount of pesticides available for exposure, of skin and/or respiratory tract, not considering protection by factors as, for example, personal protection equipment. The dose was defined as the amount of uptake into the body.

After a general introduction to the expert panel about the purposes of this study, and some general instructions, experts were provided with information on exposure to pesticides in fruit growing in the following three information phases.

Phase 1 —— A video presentation of 10 minutes described fruit growing in general and factors which may influence pesticide exposure. Additionally, methods for dermal (skin pads) and respiratory exposure assessment (personal air sampling) were shown. Next, slides were shown about activities as mixing and loading of pesticides, spraying and tasks performed in the orchards. Finally, written background information on occupational tasks in fruit growing and general information on three pesticides was given. In each phase assessments were made separately for three frequently used pesticides (*captan, azinphos-methyl and hexythiazox*). The purpose was to study if differences in physical and chemical properties of the pesticides affected rater's estimates. The pesticides represented respectively fungicides, insecticides and acaricides. Herbicides were not taken into account in this study, as they require different equipment for application and are used only infrequently.

Phase 2 —— For six randomly sampled farms from the same database as for rating *D* of orchards treated with captan, written information on dermal and respiratory exposure measurements was given together with the main farm characteristics and description of performed tasks.

Phase 3 —— Data for another six farms as described for the second phase were given. Only actual exposure data were available for captan.

Statistical analysis

Analyses were performed using Statistical Analyses System software SAS. Rankings of raters were correlated using Spearman correlation. To study inter-rater agreement, Cohen's kappa (κ) and Intra-Class-Correlation coefficient ICC (ρ), calculated by an analysis of variance (PROC NESTED), were used. PROC TTEST was used to study differences in mean exposure between ranked farms into two exposure groups.

RESULTS

Method A —— Ranking exposure according to performed tasks

The agreement between experts (ICC) varied between 0.41 and 0.81. No differences in ICC between expert groups have been observed. Generally, results were comparable for the different pesticides. For captan no differences between the information phases existed for the total group (n = 15), neither for dermal nor for respiratory exposure (Table 5). However, pesticide experts and occupational hygienists showed a small increase from phase one to three for dermal exposure. Fruit growing experts showed a small increase from phase one to three for respiratory exposure.

Table 5 Intra-class correlation (ρ) between raters in three groups of five experts in three information phases ranking 14 performed tasks in fruit growing for dermal and respiratory exposure for Captan (Method A)

		Dermal			Respiratory		
Expertise		Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
pesticide experts	ρ	0.67	0.79	0.81	0.65	0.71	0.63
	ρ_k	0.91	0.95	0.95	0.90	0.93	0.90
occupational hygienists	ρ	0.61	0.63	0.72	0.54	0.62	0.62
	ρ_k	0.89	0.89	0.93	0.85	0.89	0.89
fruit growing experts	ρ	0.76	0.72	0.65	0.53	0.63	0.65
	ρ_k	0.94	0.94	0.90	0.85	0.89	0.90
total group	ρ	0.67	0.70	0.70	0.59	0.64	0.64
	ρ_k	0.97	0.97	0.97	0.96	0.96	0.96

ρ = intra-class correlation; ρ_k = intra-class correlation as mean of k raters.

Method B —— Influence of different factors on the internal exposure dose

For all three pesticides comparable results were derived. Initial inter-rater agreement after the first information phase was higher for the estimates on spraying compared to re-entry activities (Figure 1). Per expert group some differences were observed. For fruit growing experts, initial inter-rater agreement was highest for both spraying (κ = 0.75) and re-entry activities (κ = 0.41) compared to the other experts. Information in general decreased inter-rater agreement.

Method C —— Relative contribution of exposure route to internal dose

The total group of 15 experts clearly changed their opinion after the first rating as far as dermal exposure was concerned. From the first rating phase, highest relative contribution to internal dose was attributed to the dermal route. After exposure data were presented an even higher contribution was assessed. Pesticide experts assessed the highest dermal contribution for all three tasks (mixing/loading, spraying, and re-entry) compared to the other experts. Exposure data changed the initial assessment in favour of the dermal contribution. Occupational hygienists initially assessed respiratory exposure as main contributor to internal dose. Exposure data clearly changed their assessment on that point. Dermal exposure became more important, though less explicit as assessed by the pesticide experts. The oral route is assessed as relatively more important by the occupational hygienists (contribution of around 20%) compared to the other experts, especially during re-entry of the orchard. The assessments of fruit growing experts were in between the other expert groups. From the start this group also assessed dermal route as most important. However, exposure data did not influence their assessment significantly. Mutual comparison did not show distinct differences between pesticides.

Figure 1 a

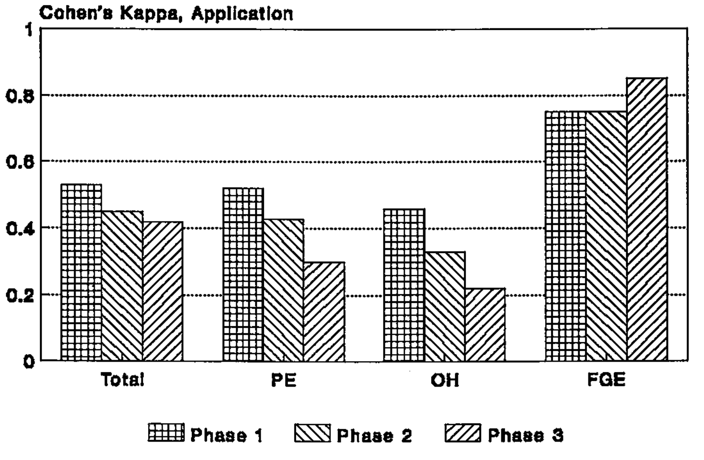
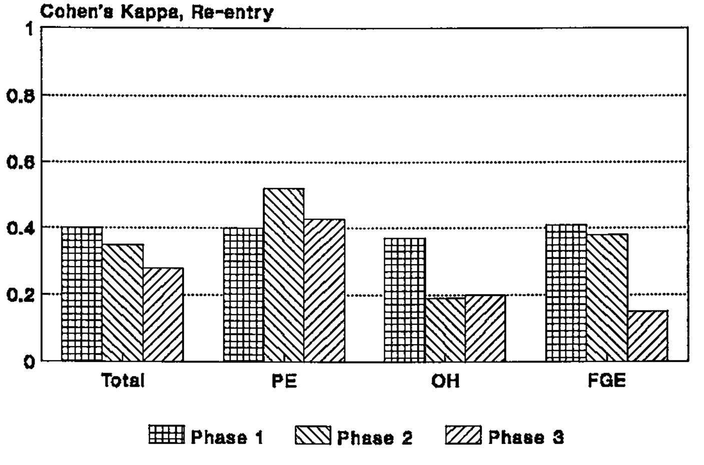


Figure 1 b



Footnote:
Total = Total group of 15 raters; PE = Pesticide Experts; OH = Occupational Hygienists; FGE = Fruit Growing Experts.

Figure 1 Inter-rater agreement (kappa) on 14 factors which may influence internal captan dose during application (1 a) and re-entry (1 b). For three × five experts, during three phases (Method B)

Method D —— Ranking of spraying activities

The intra-class correlation was higher for pesticide experts and occupational hygienists than for fruit growing experts. Highest intra-class correlation was found for occupational hygienists (Table 6).

Figures 2, 3 and 4 show the correlation between the ideal (measured) ranking and the ranking made by the experts for respiratory, total dermal exposure, and dermal exposure of the wrists, respectively. Generally, respiratory exposure was difficult to estimate. For total dermal exposure for 9 raters, and for exposure of the wrists for 7 raters the correlation between rank number and actual measured exposure was statistically significant ($P \leq 0.05$). Pesticide experts and occupational hygienists ranked spraying activities better according to actual exposure than fruit growing experts.

Pesticide experts and occupational hygienists were also able to rank farms into two groups with significantly different exposure. A statistical significant difference was found in actual measured exposure for these raters between the farms ranked as 'high exposed' compared to farms ranked as 'low exposed' (*t* test, $P \leq 0.10$, Table 7).

Table 6 Intra-class correlation (ρ) between raters in three groups of five experts for ranking of 15 spraying activities for dermal and respiratory exposure for Captan (Method D)

		Dermal		Respiratory
Expertise		total	wrists	
pesticide experts	ρ	0.43	0.41	0.75
	ρ_k	0.75	0.74	0.92
occupational hygienists	ρ	0.77	0.81	0.82
	ρ_k	0.94	0.96	0.96
fruit growing experts	ρ	0.16	0.13	0.43
	ρ_k	0.19	0.42	0.75
total group	ρ	0.40	0.43	0.67
	ρ_k	0.91	0.92	0.97

ρ = intra-class correlation; ρ_k = intra-class correlation as mean of k raters.

Table 7 Ranking of 15 spraying activities in 'high exposed' and 'low exposed' by three groups of five experts for dermal and respiratory exposure for Captan (Method D)

				Number of experts able to rank according to exposure level (*t* test) $P \leq 0.10$		
Exposure	n	AM	GSD	PE	OH	FGE
Respiratory ($\mu g\ m^{-3}$)				4	5	0
low	7	7.7	3.3			
high	8	91.6	2.0			
Dermal (total, mg hr^{-1})				3	5	1
low	7	7.1	1.7			
high	8	19.0	1.1			
Dermal (wrists, mg m^{-2} hr^{-1})				4	5	1
low	4	2.6	3.1			
high	5	14.7	1.4			

AM = arithmetic mean; GSD = geometric standard deviation;
PE = Pesticide Experts; OH = Occupational Hygienists; FGE = Fruit Growing Experts.

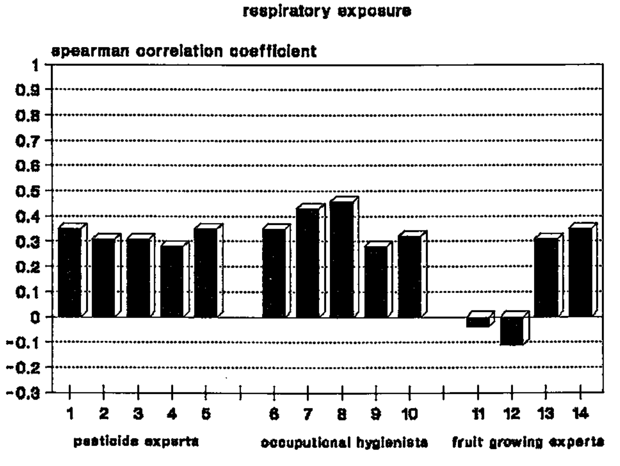


Figure 2 Spearman correlation coefficient between ideal ranking and subjective ranking for each expert for respiratory exposure (mg m^{-3}) (Method D)

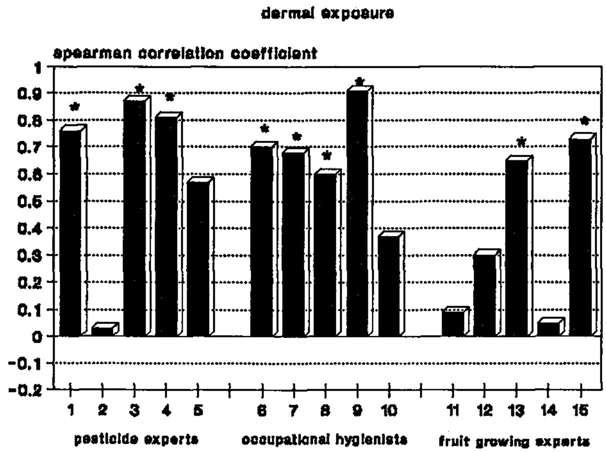


Figure 3 Spearman correlation coefficient between ideal ranking and subjective ranking for each expert for total dermal exposure (mg hr^{-1}) (Method D)

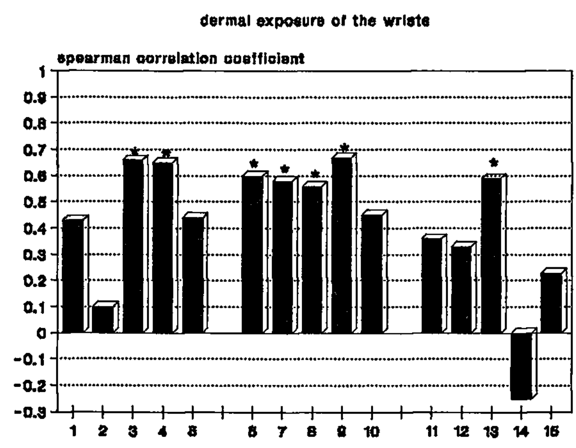


Figure 4 Spearman correlation coefficient between ideal ranking and subjective ranking for each expert for total dermal exposure of the wrists (mg m^{-2} hr^{-1}) (Method D)

* = Significant ($P \leq 0.05$)

CONCLUSIONS

Information given to experts hardly influenced inter-rater agreement on ranking tasks with respect to pesticide exposure. Perhaps the amount of information given to the experts in the second and third phase was not enough. In both phases they were provided with information of only six exposure situations. Another, more likely, explanation may have been, that the information given in the second and third phase confirmed what the experts already knew. This was supported by their ability to successfully rank 15 spraying activities according to dermal exposure.

Information on exposure decreased the inter-rater agreement when experts were asked to estimate the influence of 14 different factors on internal dose. It was observed in a study on semi quantitative estimation of exposure in a polyester factory, that relative ranking of jobs was possible without knowledge of actual exposure levels (Kromhout, 1994). It was concluded that the chemical and physical properties may influence the ability to rank from low to high. A poor comparison for styrene compared to methylene chloride was explained by differences in physical properties such as perception of smell. The study in fruit growing provided only written information on physicochemical properties to the experts. This information did not result in differences in the ratings. It should be noted that in the second and third phase only external exposure data for captan were provided without information on actual internal uptake.

In general, pesticide experts and occupational hygienists changed their estimates of the relative contribution to the internal dose of different exposure routes when provided with data on external exposure. After exposure information was provided to the experts, they judged dermal exposure more important. The occupational hygienists had initially judged the respiratory route predominant, but changed their opinion to judge dermal exposure as more important. This may be caused by their educational background, which still mainly focuses on respiratory exposure. The fruit growing experts judged the dermal route predominant from the start, probably based on their personal experiences in fruit growing.

All subgroups of experts were able to rank dermal exposure situations in a meaningful way. The results for respiratory exposure showed smaller (non significant) correlations between the ideal and the estimated ranking. The Spearman correlation coefficients for respiratory exposure were less than 0.50 and for dermal exposure for some experts around 0.90. Actual use of a cabin filter for four of the seven cabin users in this data set explained the lower respiratory exposure for these farmers very well. This was also one of the determinants of respiratory exposure which was observed in a large dataset of sprayings (de Cock *et al.* 1995a). Obviously, the experts did not consider this determinant as decisive.

Exposure data during re-entry were not taken into account for the validation of the experts' estimates, since no measurement data were available at that time. For long term exposure, however, re-entry may contribute significantly to total exposure (de Cock *et al.*, 1995b). Dislodgeable foliar residue has shown to be the most prominent determinant of exposure during re-entry among fruit growers (de Cock *et al.* 1995a). As this information is not known when estimating exposure retrospectively, one can doubt if expert panels are able to group workers retrospectively in a meaningful order. It is very unlikely that information on the concentration on the crop will be available retrospectively. Additionally, also information on re-entry times should be known.

It was striking that dermal exposure estimation was possible for single exposure situations (point estimates). Nonetheless, the crucial question for dermal exposure is which skin areas are most relevant for effects of long term exposure and whether experts are able to recognize these skin areas? Experts were able to rank total dermal exposure and exposure of the wrists. Data from a study on urinary excretion of tetrahydrophtalimide (THPI) in fruit growers exposed to captan (de Cock, *et al.* 1995c) showed the importance of dermal exposure. It was shown, however, that the highest exposed skin areas are not necessarily the most important areas for uptake. Therefore, the experts' judgement of dermal exposure may have been valid in itself, but may be invalid for retrospective assessment of uptake.

Additionally, it would be of interest for retrospective exposure assessments if experts were able to make an estimation of (dermal) exposure in the past. It is likely that exposure during applying of pesticides declined, since application techniques changed and generally became better and a cabin on the tractor was introduced to protect the fruit grower. It is not clear to what extent exposure during re-entry may have changed over the years. This may depend highly on changes in the application dose used, time spent on different tasks and time elapsed since a spraying before re-entry took place.

The use of exposure estimates of an expert panel, estimating single exposure situations, should therefore be combined with information on time spent on different tasks and information on physicochemical properties of the relevant pesticides. A prominent role of experts for retrospective evaluation of occupational exposure to pesticides in epidemiology will need further validation. Attention should focus more on the relevant measure of exposure for a specific purpose, otherwise, studies based on expert judgements may lead to highly spurious conclusions. Since experts seem to recognize the most important determinants of exposure, they may play a role in evaluating the effectiveness of control measures taken to reduce external exposure. Preferably, a panel should not be too small and consensus or average data should be used, because differences within expert groups can be considerable. The occupational hygienists and pesticide experts performed better than fruit growing experts. Therefore, also differences in expertise of expert groups may result in systematic differences.

ACKNOWLEDGMENTS

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Annex 1

Analysis of variance (PROC NESTED) was used to calculate the intra-class correlation. The reliability of the mean of k raters is defined as:

$$\rho_k = \sigma^2_\pi / (\sigma^2_\pi + \sigma^2_{\bar{\eta}}) \Rightarrow \quad \rho_k = \frac{\sigma^2_\pi}{(\sigma^2_\pi + \sigma^2_\eta / k)}$$

with σ^2_π = variance between tasks,
and σ^2_η = variance within tasks.

if $\theta = \sigma^2_\pi / \sigma^2_\eta$,

then $\rho_k = k\theta/(1+k\theta)$.

When k=1 then $\rho_1 = \theta/(1+\theta) = \sigma^2_\pi/(\sigma^2_\pi + \sigma^2_\eta)$

= variance between tasks/total variance, representing the reliablitiy of a single rater (intra-class correlation), where θ = variance between tasks/variance within tasks.

The reliability of the mean of k raters (ρ_k) is the variance due to true scores divided by the sum of the variance due to true scores and the variance due to the mean of errors of measurement.

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CHAPTER 5

Uptake of captan

CHAPTER 5.1

Urinary excretion of tetrahydrophtalimide in fruit growers with dermal exposure to captan[1]

ABSTRACT

The relation between dermal and respiratory exposure and uptake into the body of captan, measured as 24 hr cumulative tetrahydrophtalimide (THPI) dose, was studied among 14 male fruit growers applying pesticides in orchards in The Netherlands.

No contribution of respiratory exposure was observed on THPI in the urine. Dermal exposure, measured with skin pads, showed a clear relation with THPI in urine when exposure was estimated from exposure on skin pads of ankles and neck. No relation was found for total dermal exposure, calculated from measured exposure on skin pads of representative skin areas according to models described in the literature.

Determinants of exposure such as use of a cabin on the tractor, use of gloves during mixing and loading, and use of rubber boots also explained THPI in urine very well. This finding corroborated the findings on measured dermal exposure. Results indicate that more attention should be paid to skin areas which are suspected to be most permeable for a chemical under study.

It was concluded that dermal exposure data can be linked better to biological monitoring based on empirical findings as gathered in a pilot study on exposure of specific body areas than on estimations of total skin dose.

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[1] Johan de Cock, Dick Heederik, Fred Hoek, Jan S.M. Boleij, and Hans Kromhout, American Journal of Industrial Medicine 1995;28:245-256.

INTRODUCTION

It has long been known that occupational exposures may lead to dermal exposure. Probably the first measurement techniques for dermal and respiratory exposures to pesticides were described in 1962 (Durham and Wolfe, 1962). A standard protocol for dermal exposure published in 1982 forms the basis of dermal exposure assessments in field surveys of exposure to pesticides (WHO, 1982). To calculate total dermal exposure, the patch technique is used to measure a quantity on different skin locations, which is related to the total exposed body area. In a recent review of exposure assessment guidelines for pesticides, the authors observed little progress in the area of methodologies used for dermal exposure monitoring (Curry and Iyengar, 1992). Fluorescent tracer techniques and full-body dosimeters are mentioned as methods to overcome limitations of the widely used patch techniques in the future, but are still in their developmental stages.

The validity of the patch technique as a means of assessing dermal exposure rests on two critical assumptions: 1) uniform exposure, *i.e.*, the deposition rate on the patch is representative of deposition over that part of the body; or 2) worst-case exposure, *i.e.*, the patch has been located at the point of highest exposure potential for that part of the body and this exposure contributes most to the uptake. These assumptions have not been investigated systematically so that the accuracy of exposure estimates derived from this technique is unanswered (Fenske, 1993). The assumption that the quantity on a patch of known area is representative for the area of limb or other body part assuming uniformity of contamination is considered the principal limitation of the patch technique (Chester, 1993). A video imaging fluorescent tracer technique revealed nonuniform patterns of exposure which might escape detection when the patch technique is used. As a result, significant under and overestimation of exposure may occur (Fenske, 1990). In addition, extrapolation to body exposure from patches with residue levels below the limit of detection may lead to unrealistic (over)estimates, which is seen as another important drawback (Henderson *et al.*, 1993). Last, the highest exposed areas of the body, often the hands, need not contribute significantly to total uptake, thus making the worst-case approach doubtful.

Evaluation and control of dermal exposure are hindered by the lack of suitable methods for measurement of dermal absorption in humans, inter species differences in skin permeability, regional differences in absorption rates, skin damage, and exposure conditions in the workplace (Fiserova-Bergerova, 1993). The relevance of surface area relies on accurate and correct measurement. Slone (1993) disputed both, and suggests relying on empirical evidence of toxicity and/or metabolism of chemical exposure, which is not dependent upon surface area.

Biological monitoring is mentioned to overcome many of the limitations of passive dermal dosimetry, but extensive knowledge of the pharmacokinetics and metabolism of the pesticide is needed before this approach can yield interpretable information (Curry and Iyengar, 1992). Biological monitoring offers the best means of accurately assessing human exposure, since it assesses actual, rather than potential, absorption.

Large inter species differences in metabolism and the higher doses used in animal studies compared to human exposure, however, make it difficult to extrapolate to human metabolism from animal metabolism studies, and individuals may differ in their metabolism and excretion of the compound (Woollen, 1993).

Inaccurate estimates of exposure to reach safety and regulatory decisions that can result from less direct methods of exposure assessment should be avoided. As an example, a study of Maddy *et al.* (1989) showed that metabolite measurements in urine were considerably lower than predictions of human exposure based on external exposure measurements for exposure of applicators and harvest workers to captan in strawberry fields.

Research on associations between dermal exposure in sprayers and absorption of pesticides often has shown poor results (Fenske, 1988; Chester and Hart, 1986; Franklin *et al.*, 1981). However, in a recent study (Brouwer *et al.*, 1993) the relation between exposure to propoxur and 2-iso-propoxyphenol (IPP) was studied and showed a distinct correlation between dermal as well as respiratory exposure with IPP in urine during harvesting of carnations. Dermal exposure of hands and forearms was estimated from dislodgeable foliar residue and total number of working hours.

As part of a research program on pesticide exposure in fruit growing, a study was carried out on the relation between external dermal and respiratory exposure to captan and the metabolite tetrahydrophtalimide (THPI) in urine. THPI is suggested as a relatively simple biological monitoring model. It assumes measurement of THPI as a principal, stable captan metabolite (van Welie *et al.*, 1991). THPI is used as measure for uptake of captan into the body.

In this study, the contribution of dermal and respiratory exposure to THPI in urine was studied among fruit growers applying captan in orchards in The Netherlands. To derive exposure estimates for epidemiologic purposes, estimates of total dermal exposure were based on extrapolation to total body exposure from skin pad exposures and compared to estimates based on exposure of skin pads of specific body areas. Also the influence on THPI in the urine of specific determinants of exposure, such as personal protection and equipment used, was studied.

MATERIALS AND METHODS

Study population

A group of 26 male fruit growers with a mean age of 42 years (± 13) and weight of 85 kg (± 11) participated in a study on the relation between external exposure to captan and uptake into the body during applying in the orchard with an airblast sprayer pulled by a tractor. This study is part of a research program on pesticide exposure in fruit growing, which is described in more detail elsewhere (de Cock *et al.*, 1994a). In urine samples of 18 fruit growers, randomly selected from the participants, THPI, a metabolite of captan, was analyzed. The relation between THPI dose and external exposure and work characteristics was examined. Due to differences in the urine analysis for three subjects, only data of 15 subjects were used. Additionally one subject was excluded because not all urine excreted during a 24-hr period was sampled.

Exposure measurements and analysis

For sampling pesticide particles, a personal air sampling head (diameter 6 mm) with glassfiber filter (diameter 2.5 cm Whatman, GF/A) was used resembling the inhalable dust fraction (Smid *et al.*, 1992), connected to a portable pump device (DuPont

P2500) with a flow of 2 L/min. To measure dermal exposure, the patch technique was used. The term (skin)pad will be used. Skin pads made of α-cellulose (Schleicher and Schüell), diameter 3.5 cm, were used and placed between two circular Fixomull stretch bandage plasters (BDF), diameter 5 cm, with a circular opening of 2.5 cm in the upper plaster layer. Body locations for the skin pads were based on the standard protocol of the WHO (1982, 1986). Pads were applied directly on the skin. Extraction from the airsample filter and skin pads took place by adding an acetonitrile/water mixture (50/50%) and vibration in an ultrasonic bath during 5 min. The analysis of skin pads and air samples was carried out using high performance liquid chromatography (HPLC) (de Cock *et al.*, 1994a). The limit of detection was 17 μg/L, which is equivalent to 12.8 ng cm^{-2}.

THPI analysis

THPI in urine was used as biological monitor (van Welie *et al.*, 1991). A blank urine sample was taken the evening before the fruit grower started applying captan. The subjects started work in the orchard the next morning with an empty bladder. Every urine sample during the following 48 hr was collected in separate bottles. THPI at 24 and 48 hr was calculated by adding up THPI levels from separate samples for the 24 and 48-hr period, respectively. THPI was analyzed with chemical ionization (CI) and mass spectrometry (GC-MS). The limit of detection was 2.7 μg/L. Analysis was carried out at the Free University of Amsterdam using a method described by van Welie *et al.* (1991).

Pad locations

Pads, 13 in total, were placed on the front side above the ankles (left, right), below the knee (left, right), on the mid thigh (left, right), forehead (two, side by side), inner side of the wrists (left, right), on the back between shoulder blades, the front ("V") of the neck, and on the back of the neck. Pads on head, wrists, and neck were not covered by clothing. Wrist pads could partly be covered when gloves were used.

Calculation of exposure

For the analyses, total skin exposure was calculated based on the standard protocol of the WHO (1982) and a method described by Popendorf and Leffingwell (1982). Formulas:

WHO (mg) = 10^{-3}×150×[V of neck)] + 1100×[back of neck+head (left+right)]/3 + 2000×[left wrist], with the amounts of individual pads in μg, representing 3,250 cm^2 (±18% of total body area). Popendorf and Leffingwell (mg) = 10^{-3}×115.2×[V + back of neck)]+ 1075.2×[head (left+right)]/2 + 1075.2×[wrist (left+right)].

In addition to these models estimating total dermal exposure, analyses were also carried out with individual skin pad exposures (μg cm^{-2}) or the mean of a combination of skin pad exposures of a specific body area. The inhalable captan concentration (mg m^{-3}) was used as an estimate of respiratory exposure. Calculations for the total skin exposure dose, based on the WHO model, were made for skin not covered by clothing. Exposures of covered pads were nondetectable or very low and would have led to unrealistic calculations.

Statistical analysis

All analyses were performed using Statistical Analysis System software (SAS). The exposure distribution was tested using PROC UNIVARIATE. PROC TTEST was used to compare subgroups, PROC REG was used to study the relation between THPI in urine and determinants of exposure in univariate and multivariate analyses. A two sided *P*-value of 0.05 was used as a criterion for statistical significance.

RESULTS

In Table 1, data on dermal and respiratory exposures and on THPI levels in urine are presented for the total group, and broken down into use of a cabin on the tractor, wearing of gloves during mixing and loading, and use of rubber boots. Inhalable captan exposure did not exceed the threshold limit value (TLV) of 5 mg m^{-3} (ACGIH, 1992). Highest dermal exposure was found on the forehead, followed by the wrists and neck.

It was not possible to pronounce upon use of glasses and masks nor on socks and trousers because they were hardly worn or because they were worn by all farmers. In most cases an undershirt with shirt were worn and three farmers only wore a shirt. Often also a sweater was worn. Overalls and spraying suits were equally used. Two farmers wore a rain suit on a tractor without a cabin. For the subjects without a cabin on the tractor, THPI in urine was higher. Farmers who used gloves during mixing and loading (n = 7) had a lower level of THPI in urine. A striking finding was that THPI was significantly lower for subjects wearing rubber boots.

Average spraying duration was 211 min with a standard deviation of 84 min. The amount of captan handled per spraying varied from 1.7 to 22.5 kg (mean 10.4 kg). The crop area sprayed was 5.9 hectare (± 3.0), and the amount sprayed per hectare was 1.2 kg/hectare (± 0.43). Because results based on 24-hr urine were comparable with 48-hr urine and urine was not sampled correctly for all subjects for the complete two days, results for 24-hr THPI were used. Univariate regression analyses revealed no significant relation between respiratory exposure and THPI levels. Skin exposure of the neck and the forehead and, to a lesser degree, exposure of the ankles (not significant) was related to THPI in urine. Neck and ankles were not the highest exposed areas (Table 2). For the ankles in eight subjects, exposure was under the limit of detection. Measured dermal exposure on the ankles indicated a lower, but not significant (Chi square: $P=0.26$) exposure for rubber boot users (0.016 $\mu g\ cm^{-2}$ for wearing, of whom five subjects < limit of detection, and 0.028 $\mu g\ cm^{-2}$ for not wearing, respectively). No significant relation was found for exposure of the wrists. Total dermal exposure as calculated by the WHO model was not related to THPI ($P=0.43$), neither was dermal exposure based on the model proposed by Popendorf and Leffingwell (1982; $P=0.15$).

Table 1 Dermal and respiratory exposure to captan and THPI in urine of Dutch fruit growers according to exposure characteristics, The Netherlands, 1990

	n[a]	AM[b]	GSD[c]	Range[d]	
Captan exposure[e]					
Inhalable fraction (mg m^{-3})	13	0.13	3.9	0.009-0.70	
Dermal exposure (μg cm^{-2})					
Forehead	14	2.48	11.7	0.006-14.87	
Wrists	14	1.79	4.8	0.04-8.22	
Neck (V+back)	14	0.27	5.3	0.006-1.41	
Uncovered skin[f]	14	1.51	5.2	0.07-6.43	
Ankles[g]	14	0.02	2.7	0.006-0.095	
WHO model (mg)[h]	14	4.22	4.0	0.29-14.1	
THPI in urine (μg)[i]					
24 hr	14	11.5	2.4	2.3-26.7	
48 hr	13	18.4	2.4	2.9-45.1	
THPI (μg,24 hr)					*P*-value[j]
Cabin on tractor					
No	6	16.3	2.3	3.7-26.7	
Yes	8	7.9	2.3	2.3-22.3	0.10
Gloves during mixing/loading					
No	7	14.8	1.9	5.9-26.7	
Yes	7	8.2	2.6		0.07
Protection ankles (rubber boots)					
No	7	18.1	1.9	6.0-26.7	
Yes	7	5.0	1.8	2.3-9.5	0.002

[a]Number of observations; [b]Arithmetic mean; [c]Geometric standard deviation; [d]Range; [e]Except for the variable uncovered skin and ankles, exposure variables were log normally distributed; [f]Mean skin exposure (μg) of 6 uncovered skin pads of head(2) neck (V,back), and wrists (2); [g]8 observations under limit of detection (LOD) and set to 0.5 times LOD (=25.8 ng); [h]AM of 4.22 mg = 1.30 μg cm^{-2} (based on 3,290 cm^2 exposed skin). WHO (mg) = $10^{-3}\times150\times$[V of neck)] + $1100\times$[Back of neck+head (left+right)]/3 + $2000\times$[left wrist]; [i]THPI in urine (μg) was log normally distributed; [j]*P*-value of *t* test for log-transformed concentration in urine.

Table 2 Univariate linear regression analysis of exposure characteristics related to log-transformed 24-hr THPI dose (μg) of 14 fruit growers after spraying orchards with captan, The Netherlands, 1990*

	Regression coefficient	Standard error	P-value
Inhalable fraction (mg m^{-3})	-1.20	1.16	0.33
Uncovered skin[a]	0.200	0.105	0.08
Wrists	0.060	0.095	0.54
Forehead[b]	0.093	0.047	0.07
Neck (V+back)[c]	1.27	0.534	0.03
Neck (back)	1.28	0.644	0.07
Ankles	13.6	7.99	0.12
WHO (mg)[d]	4.29 10^{-5}	5.3 10^{-5}	0.43
Popendorf (mg)[e]	4.00 10^{-5}	2.62 10^{-5}	0.15
Cabin (0/1)	-0.79	0.446	0.10
Amount captan (kg)	-0.020	0.04	0.65
Spraying time without cabin (min)	0.003	0.0014	0.03
Gloves during mixing/loading (0/1)	-0.855	0.430	0.07
Ankles protected (0/1)[f]	-1.30	0.323	< 0.01
Age of fruit grower (year)	0.004	0.020	0.84

*Dermal exposure in μg cm^{-2}; [a]Mean skin exposure (μg) of six uncovered skin pads of head(2) neck (V,back) and wrists (2); [b]Means of two pads on forehead (μg cm^{-2}); [c]Skin pads on V and back of the neck (μg cm^{-2}); [d]Skin exposure WHO (mg) = $10^{-3}\times150\times$[V of neck)] + 1100×[Back of neck+head (left+right)]/3 + 2000×[left wrist] (WHO,1982); [e]Skin exposure POP (mg) = $10^{-3}\times115.2\times$[V + back of neck)]+ 1075.2×[head (left+right)]/2 + 1075.2×[wrist (left+right)] (Popendorf and Leffingwell,1982); [f]Protection of ankles with rubber boots yes(1) or no(0).

A positive and borderline statistically significant relation (P=0.08) was found between the average of the sum-value of the exposure of all six skin pads not covered by clothing and THPI levels. Of the potentially protecting factors, cabin on the tractor, use of gloves during mixing, and loading of captan were negatively associated with THPI levels. A very strong negative relation was found for wearing rubber boots. The use of a cabin was negatively associated with THPI in urine, with a P-value of 0.10, on the borderline of significant. A clear relation with higher THPI dose was observed with spraying time when a cabin on the tractor was absent. Finally, no relation was found with amount of captan handled or with age of the subjects.

Because of the strong relation between THPI in urine and the exposure of the neck area and the potentially protective factors, the effect of wearing of rubber boots, dermal exposure of the ankles, and spraying time without a cabin were studied in more detail. In Table 3, multivariate analyses are presented for 24-hr THPI in urine. The regression coefficients represent the estimated change in ln(THPI) per change in units of the independent variables. Exposure of the mean of front ("V") and back of the neck and ankles explained 58% of the variation in THPI. Results based only on the pad on the back of the neck were comparable (data not shown). When use of rubber boots was used instead of actual measured ankle dose, 67% of the variance was explained. Comparable results were obtained in a model with spraying time without a cabin and rubber boots as independent variables (69%). Because of a strong mutual relation between skin exposure and cabin use, these two variables were not put together in one multivariate model. Also the effect of using gloves during mixing and loading together with the use of rubber boots showed a high explained variance of 73% (data not shown). In a model with the three main determinants of potential dermal protection (cabin, gloves, and rubber boots), cabin on a tractor did not contribute to explained variance in the model indicating that gloves and rubber boots were the major determinants to protect farmers. Dermal exposure of the neck and using gloves and rubber boots as independent variables in a multivariate regression analysis explained the variance in THPI dose (R^2 = 80%) very well. When the adequacy of this model was tested by residual analysis, one outlier was detected. Removal improved the strength of the relation of the independent variables with THPI as well as explained variance (data not shown).

Despite the small number of subjects, the multivariate regression models showed stable regression coefficients and standard errors when independent variables were interchanged. This is illustrated in the last two models in Table 3, where cabin on a tractor in one, and exposure of the neck in another model are put into a multivariate analysis together with use of gloves and rubber boots. The regression coefficients and standard errors of the variables gloves and rubber boots were comparable in both models. In Figure 1, the relations between measured and predicted THPI in urine according to neck dose, use of gloves, and rubber boots are presented. Because of the small numbers and minor effect on the results, the one outlier was not removed. Respiratory exposure did not explain any variance in THPI in any of the multivariate analyses.

Table 3 Multivariate linear regression analysis of exposure characteristics related to log-transformed 24-hr THPI dose (μg) of 14 fruit growers after spraying orchards with captan, The Netherlands, 1990

	Regression coefficient	Standard error	*P*-value	Explained variance R^2
Intercept	1.38	0.252	< 0.01	0.58
Neck (V + back) (μg)	1.40	0.443	0.01	
Ankle (μg)	15.7	6.074	0.03	
Intercept	3.14	0.244	< 0.01	0.75
Cabin on tractor (0/1)	-0.28	0.296	0.37	
Gloves during mixing/loading (0/1)	-0.61	0.283	0.06	
Ankles protected (0/1)	-1.13	0.283	< 0.01	
Intercept	2.76	0.254	< 0.01	0.80
Neck (V + back) (μg)	0.64	0.345	0.09	
Gloves during mixing/loading (0/1)	-0.62	0.248	0.03	
Ankles protected (0/1)	-1.03	0.262	< 0.01	

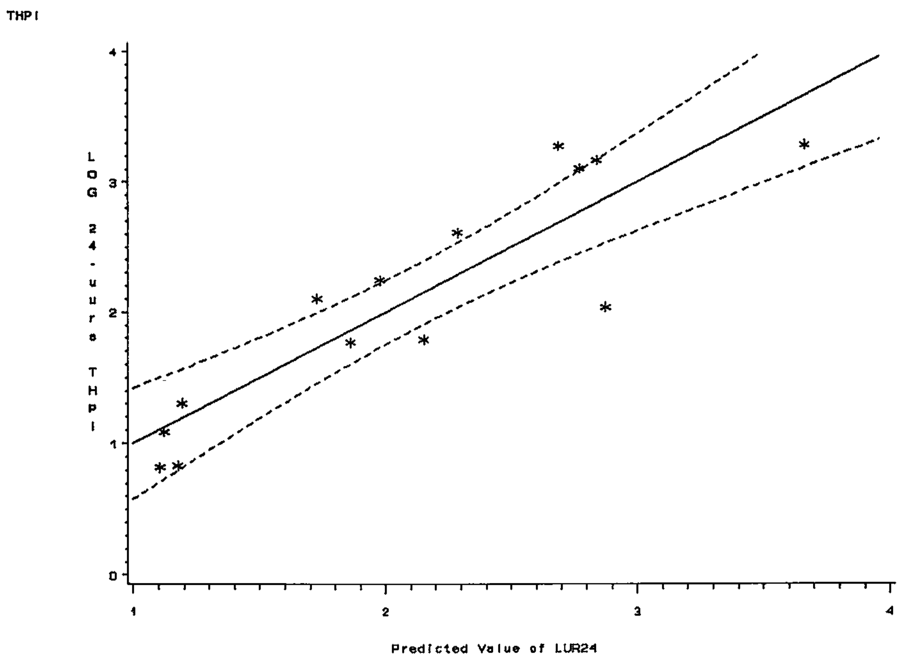

Figure 1 The relation between log-transformed 24-hr THPI dose in urine and the predicted THPI level in urine, based on a regression model including dermal exposure of the neck (V and back), wearing of rubber boots and gloves for 14 fruit growers after spraying orchards with captan, The Netherlands, 1990

DISCUSSION AND CONCLUSIONS

Dermal exposure to captan measured with skin pads was clearly related to 24-hr THPI levels in urine. No relation was observed for respiratory exposure. Protection of the skin by using a cabin on the tractor and wearing rubber boots resulted in a lower uptake of captan. Dermal exposure of the neck area, based on absolute amounts on the pads and protection of the ankles, explained very well THPI in urine, which was also true for, and seems consistent with, a model with exposure variables spraying time without a cabin and rubber boots use. The relation of THPI in urine with these determinants of dermal exposure supports the idea that the skin is the major route of absorption and that there is an apparent contribution of specific body areas. Additionally, use of a cabin and wearing of rubber boots and gloves could be variables representing awareness of exposure and could be markers of differences in hygienic behaviour in general, resulting in a lower final uptake.

Depending on the application techniques used, relatively large differences in the distribution of pesticides over the body have been observed (van Hemmen, 1992, 1993). Here, highest exposure was found on the forehead, followed by the hands. Mostly, hygienic behaviour only includes washing of hands and sometimes the face directly after spraying has finished. Availability for absorption is an important factor influencing absorption of chemicals. This may be of importance especially for neck and ankles, because these skin areas are often not washed directly after a work shift. On the other hand, it is doubtful if washing removes captan completely from contaminated skin (Fenske *et al.*, 1991; de Cock *et al.*, 1994a). The absolute amount on the ankles was low compared to other skin regions. For eight subjects no detectable amount of captan was found on the ankle pad, but wearing rubber boots probably results in protection of a larger area of foot and lower leg. The skin pads were placed on the front side above the ankle. It also seems realistic to assume higher exposure in case of wearing shoes or clogs, because contamination of the inside could easily occur. Besides, rubber boots used for spraying more often are changed directly after spraying than shoes and clogs (personal observation).

Important factors which determine percutaneous absorption of chemicals are reviewed by Grandjean (1990) and include exposure factors such as concentration and type of vehicle, extent of skin contact (*i.e.*, the area covered), duration and efficiency of removal. Other factors concern the skin, such as regional variability of skin permeation, skin disease and wounds, and skin hydratation in combination with recent contact with damaging chemicals. Occlusion and temperature are also important.

According to data of Scheuplein and Bronaugh (Grandjean, 1990), generally penetration rate will vary according to the skin site involved. A ranking was given from the plantar with highest penetration rate followed by scrotum, palms, dorsum of hands, forehead and scalp, to arms, legs and trunk with lowest rate. It is suggested that regional differences may occur according to the chemical involved. As an example that differences do occur, a study of Maibach *et al.* (1971) was cited. This study shows that malathion penetrated faster through horny pads then through the scalp, and fastest through scrotal epidermis. For captan, our study revealed a clear relation between dermal exposure of certain areas with 24-hr THPI level in urine. The strength of this relation is expressed as the regression coefficients for the independent variables in the regression models. These coefficients comprise the influence of a number of factors as level of exposure, uptake through the skin, and metabolism. Which of these factors is most prominent, cannot be elucidated. On the basis of these regression models, however, the relative importance of different skin areas is illustrated by the regression coefficients in Table 2 for the ankles (13.6) compared to the forehead (0.093). Despite the fact that exposure of neck and ankles was lower compared to forehead and wrists, differences in permeability and availability for uptake may have accounted for the observed relation between these skin areas and THPI in urine. Interestingly, exposure of the wrists did not contribute to THPI in urine.

Results of this study indicate that more attention should be paid to skin areas which are suspected to be most permeable for a chemical under study, instead of estimation of total skin exposure. Emphasis should not be put on areas with highest absolute exposure only. This is illustrated by the observation that the models, including exposure estimates based on WHO and Popendorf for total dermal exposure, were not significant. An explorative study on time to pregnancy (TTP) and pesticide exposure among fruit growers showed an association between TTP and determinants relating to dermal exposure (de Cock *et al.*, 1994b). Exposure was measured on forehead, back of the neck, wrists and arms, but other skin areas with a higher penetration rate may be of interest too. For epidemiologic studies on reproduction, for instance, the skin of the scrotum may be of special interest in this respect.

It is impossible to estimate permeability of the skin for captan based on this study. However, captan primarily is absorbed through the skin and contributes to actual uptake. Contribution of the respiratory exposure route on cumulative THPI level is of minor relevance here. Since no human data on excretion kinetics are available, it is impossible to pronounce upon possible health risks for fruit growers applying captan at the measured dose more accurately.

In general, dermal exposure gains in significance as exposure route when permissible occupational inhalation exposures are lowered (Fiserova-Bergerova, 1993; Fenske, 1993). A criterion based on comparison of the dermal penetration rate with pulmonary uptake rate at inhalation exposure permissible in the workplace was advocated to replace the current practice of using acute dermal toxicity (LD50) as a warning sign for significant dermal absorption (Fiserova-Bergerova, 1993). Our study indicates, however, that dermal absorption occurs without an obvious contribution of the respiratory route. Furthermore, it is recognized that it is very difficult to measure the contribution of the oral route directly, but should not be overlooked (Woollen, 1993). It is not possible to elucidate the role of the oral route, but based on the high explained variance in our study, the dermal route seems predominant. As an alternative explanation, however, it is possible that, indirectly, dermal exposure is related to oral exposure.

Because many factors potentially influence actual absorption through the skin, dermal exposure data better can be linked to biological monitoring measured by empirical observations than on an estimation of total skin dose, for each specific chemical and exposure situation. This is supported by the finding that total dermal exposure based on projection of measured exposure to represented skin areas of specific skin pads did not explain THPI in urine, in contrast with the findings based on specific skin areas only. Because differences in skin permeability may vary for different chemicals, it is impossible to generalize these findings to other chemicals or exposure situations. Therefore, first pilot studies are necessary to clarify which determinants of exposure are important in each case.

Based on this study, use of skin pads should be considered a useful tool for defining exposure groups according to uptake and for determining factors of the workplace, which explain exposure. Although use of the patch technique can locally prevent dermal uptake of pesticides, which may influence the relation with a biological monitoring measure, this effect can be minimized if pads are not too large.

As mentioned, uniformity of dermal exposure is an important issue. In general, personal monitoring is highly dependent on body location where the sampler is placed. A study on measurement on airborne iron oxide revealed a 36-71% lower concentration within welding helmets compared to the concentration outside (Goller and Paik, 1985). From data presented by Goller and Paik, we calculated a coefficient of variance of 25% between helmet and chest and 22% for chest, left, and right shoulder. For air pollution exposure assessment, it is common knowledge that this error is relatively small compared to differences in exposure between workers and from day to day. In our study, the ratio of the 97.5th and 2.5th percentiles ($\hat{R}_{0.95}$) of the log-normal exposure distribution for dermal exposure of the head showed an extreme value of more than 15,000. Also for wrist and neck area this ratio was very high (468 and 690, respectively). Comparable results are not unusual for respiratory exposure for some tasks (Heederik *et al.*, 1991). Because no repeated measurements were available, it is not known if the observed differences, expressed as $\hat{R}_{0.95}$'s, can be attributed to systematic differences between farmers (inter-individual variability) or day-to-day fluctuations (intra-individual variability). It is interesting to note that the sampling error and analytical error for the head pad appeared to be 57%, expressed as coefficient of variation, based on duplicate pads. This error is of marginal importance given the exposure distribution resulting in differences in exposure up to a factor of 15,000.

This might lead to the conclusion that there seems to be some overemphasis on the sampling error made with dermal pads because of inhomogeneity of exposure of the skin area. For instance, for epidemiologic purposes misclassification of individuals with respect to their exposure due to the sampling and analytical error when using skin pads seems minimal. For epidemiologic studies it seems more useful to concentrate on the major sources of exposure variability like inter- and intraindividual variability of exposure, by using an approach of repeated measurements to estimate within- and between-farmer variability. Based on the results in our study one might decide to limit exposure assessments to the skin patch areas of neck and ankles for large epidemiologic research on applying of captan in fruit growing.

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CHAPTER 5.2

Strategy for assigning a "skin notation": a comment[2]

INTRODUCTION

For occupational exposure, the skin may play an important role as route for uptake into the body. In general, dermal exposure increases in significance as exposure route when permissible occupational inhalation exposures are lowered (Fiserova-Bergerova, 1993; Fenske, 1993). A criterion based on comparison of the dermal penetration rate with pulmonary uptake rate at inhalation exposure levels permissible in the workplace was advocated to replace the current practice of using acute dermal toxicity (LD50) as a warning sign for significant dermal absorption (Fiserova-Bergerova, 1993).

When percutaneous absorption potentially has a significant contribution, in most occupational exposure lists, a "skin notation" is added (Boleij *et al.*, 1995). The criteria that lead to a "skin notation" however are generally not specified. The purpose of this notation is to indicate the need to prevent skin contamination when systemic effects (not local effects) may result from percutaneous absorption of the material as a gas, a solid or a liquid (ECETOC, 1993a).

The provisional approach by the Dutch Expert Committee on Occupational Standards (DECOS) implies that a skin notation should be applied when the amount absorbed by both arms and forearms in one hour could amount to more than 10% of the

[2] Johan de Cock, Dick Heederik, Hans Kromhout, Jan S.M. Boleij. Strategy for assigning a "skin notation": a comment. Submitted for publication.

amount absorbed via the lungs on exposure to the occupational exposure limit (OEL) for 8 hours. It is assumed here that the OEL is based on systemic toxicity. It is recognized by ECETOC that this approach is hampered by the fact that little data is available in the literature concerning absorption.

When there is lack of data, an empirical formula as a helpful tool for warning on the potential of significant dermal absorption was suggested by Fiserova-Bergerova (1993). A dermal penetration rate (flux), predicted from physical properties of 132 chemicals as an index of dermal absorption potential was suggested.

In a study on urinary excretion of tetrahydrophtalimide (THPI) in fruit growers with dermal exposure to captan (de Cock *et al.* 1995a), we showed recently a clear relation with THPI in urine when exposure was estimated from exposure of the ankles and the neck. Our study indicated, that dermal absorption occurred without an obvious contribution of the respiratory route. Here we will consider the consequences of our findings in relation to the current criteria for setting a "skin notation".

METHODS

The documentation on a strategy of assigning a "skin notation" (ECETOC, 1993b) assumes an absorption rate R of

$$\mathbf{R = (10^{b}\ [m^{3}] \times OEL\ [mg/m^{3}]) \times f^{c}\ /\ (2000^{a}\ [cm^{2}] \times 10^{d})}$$

where the area of the hands and the forearms is [a]2000 cm^2, that [b]10 m^3 is inhaled in 8 hours, that a fraction (f) of [c]0.5 of the atmospheric contaminant (assumed by default) is absorbed by the lungs, at the [d]10% criterion of DECOS.

Thus, if $R > 0.25 \times$ OEL [$\mu g/cm^2$] a "skin notation" may be appropriate. For captan with a OEL of 5 mg/m^3 and no "skin notation" (ISZW, 1995) the criterion for a "skin notation" would be 1.25 $\mu g/cm^2$ per hour.

The empirical formula for the skin penetration J (mg cm^{-2} h^{-1}) as described by Fiserova (1993) is:

$$J = C_{sat}/15 \times (0.038 + 0.153\ P_{ow})e^{-0.016MW}$$

with C_{sat} = the aqueous solubility (mg/L); P_{ow} = octanol-water partition coefficient; MW = Molecular Weight; For captan J= 0.1674 μg cm^{-2} h^{-1}, based on C_{sat} = 3,3 mg/L = 3.3 10^{-3} mg/ml at 25 Celsius; P_{ow} = 610 at 25 Celsius; MW = 300.61 (Worthing, 1991).

RESULTS

Based on the empirical formula of Fiserova (1993), skin penetration would be 0.17 μg cm^{-2} per hour, which is not larger than 1.25 μg cm^{-2}. Following this approach captan would not have a "skin notation".

The inhalable dust fraction in our study (de Cock, *et al.* 1995a) of 0.13 mg/m^3 was definitely lower than the OEL (5 mg/m^3). Dermal exposure for the hands and forearms based on our data of the wrists of 1.79 μg cm^{-2} for an average exposure of 211 min, would result in 0.51 μg cm^{-2} per hour and not lead to a "skin notation". As we found a clear relation with THPI in urine for the neck (1100 cm^{-2}) and ankles (2300 cm^{-2}), these skin areas are of specific interest. A calculation for these areas results in an external exposure of $((0.27^a \times 1100) + (0.02^b \times 2300))/(1100+2300) = 0.10$ μg/cm^2 for 211 min, which is 0.03 μg cm^{-2} per hour, where [a]0.27 is exposure of the neck and [b]0.02 exposure of the ankles (de Cock, *et al.* 1995a). Striking in this perspective is that for the skin area of neck and ankles, which explained THPI in urine very well, the external dose was 17 times (0.03/0.51) lower than for the hand-arm area.

DISCUSSION AND CONCLUSIONS

Based on our findings, at least for captan, the approach for setting a "skin notation" based on an occupational exposure limit for respiratory exposure and exposure of the hands and the forearms does not seem applicable. The dataset was small (only 14 fruit farmers), but it points at the possible differences in contribution of different skin locations to uptake.

Based on the empirical formula of Fiserova-Bergerova (1993) and our findings, following the ECETOC strategy, a "skin notation" does not seem necessary for captan. At this moment the OEL for captan is being reconsidered in The Netherlands (ISZW, 1995). If the OEL had been less or equal 2 mg/m^3, then following the ECETOC-strategy, captan would have had a "skin notation", but not based on the most relevant skin sites for dermal absorption.

One can doubt if a "skin notation" based on a OEL as used by DECOS is a good starting point, since we experienced that the much lower absolute exposure of the neck and ankles contributed to THPI in urine, without a clear contribution of dermal exposure of the wrists and of the respiratory route. Furthermore, and more important, since the OEL is set on the basis of data for respiratory exposure, one can argue that in a situation where the dermal route predominates and respiratory exposure is of minor importance, the OEL is not a valid starting point to derive a "skin notation" in itself. In such a case a separate standard for dermal exposure seems more appropriate.

ECETOC state in their document that it is recommended that this harmonized approach should be reviewed in the light of experience in use, any formal validation undertaken and any scientific or technical progress (ECETOC 1993b). Viewed in that light, we hope that future focus will be put on the most relevant skin sites of exposure and not only on the hands and arms. An important aspect of dermal exposure is, that contamination of the skin often will be available after the workshift, where respiratory exposure stops. Besides, contaminated hands may play a role in a more indirect sense as a secondary source of contamination for other parts of the skin.

We concluded that for studies on the contribution of dermal exposure to uptake our results indicate that more attention should be paid to skin areas which are suspected to be most permeable for the chemical under study and that emphasis should not be put on areas with highest absolute exposure only. Future research should focus on variation in skin permeability over the human body for different chemicals. As work practices in agriculture may differ highly from those in other industries with respect to exposed body sites, a similar strategy for both situations may not be applicable in all cases.

It might be worth considering the suggestion of Fenske (1993) concerning developing dermal occupational exposure limits (DOEL). It was suggested that DOEL's might be feasible for selected workplaces and environmental agents. These limits are based on surface sampling. However, in a study on exposure to pesticides during re-entry of fruit orchards, we found only a clear relationship with dislodgeable foliar residue for some skin areas and also a contribution of the airborne concentration on dermal exposure (de Cock *et al.* 1995b). Therefore, developing DOEL's will need some further exploration.

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CHAPTER 6

Time to pregnancy and occupational exposure to pesticides in fruit growers in The Netherlands[1]

ABSTRACT

Although pesticides are regularly used in agriculture, relatively little is known about possible adverse health effects, especially reproductive effects, due to occupational exposure. This explorative study investigates the relation between exposure of the fruit grower to pesticides and fecundability (probability of pregnancy) in a population of fruit growers.

The analysis is based on self reported data and includes 91 pregnancies during 1978-1990 of 43 couples. Cox' proportional hazards model was used to analyse time to pregnancy after correction for gravidity and consultation with a physician for fertility problems.

Application of pesticides solely by the owner was associated with a long time to pregnancy, resulting in a fecundability ratio of 0.46 (95% confidence interval (95% CI) 0.28-0.77). Similarly a low spraying velocity (≤ 1.5 hectares/h) resulted in a

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fecundability ratio of 0.47 (95% CI 0.29-0.76) and is associated with the use of older spraying techniques and tractors without a cabin. These factors were assumed to cause high exposure, which was confirmed by exposure measurements in the field. The effect of high exposure was mainly apparent if the couple had intended to become pregnant in the period from March-November (fecundability ratio 0.42, 95% CI 0.20-0.92). This is the period in which pesticides are applied. Out of the spraying season the effect of a high exposure was absent (fecundability ratio 0.82, 95% CI 0.33-2.02). In the high exposure group 28% of the pregnancies had been preceded by consulting a physician because of fertility problems, compared to 8% in the low exposure group. These findings indicate that an adverse effect of exposure to pesticides on fecundability is likely.

INTRODUCTION

Based on some case reports, physicians of the Academic University Hospital of Utrecht in The Netherlands suspected a link between use of pesticides and infertility among men who visited the fertility unit of the clinic. These indications were not substantiated by the scientific literature.

Baird *et al.* (1986) suggested the use of time to pregnancy as a simple measure in epidemiological studies to find the effects of environmental exposures on fecundability. According to Zielhuis *et al.* (1992) valid data on time to pregnancy can be provided by a face to face interview. Time to pregnancy is the number of non-contraceptive cycles that it takes a couple to conceive, or the number of menstrual cycles expressed in months. The fecundability of a couple, the probability of conception for each menstrual cycle, is estimated by the inverse of time to pregnancy. Fecundability is dependent on many different biological processes, including gametogenesis, transport of germ cells, fertilisation, transport of the embryo, implantation, and early survival of the foetus. Toxic substances may interfere with these processes through a range of different mechanisms (Kiely, 1991; Mattison 1983). Therefore, time to pregnancy is a good measure to take when no specific hypothesis can be formulated. It can be used to study reproductive effects in men. Furthermore, small effects within the normal individual variation may be discovered, and an additional advantage is that data on time to pregnancy can be obtained by a simple questionnaire (Baird *et al.*, 1991). So far, mostly semen analysis has been used to examine the effect of specific agents on male fertility (Rosenberg *et al.*, 1987; Wyrobek *et al.*, 1983).

Most studies on pesticides describe toxicological effects on spermatogenesis of dibromochloropropane (DBCP) (Whorton *et al.*, 1977; Sandifer *et al.*,1979; Glass *et al.*,1979; Schrader *et al.*, 1987) and to a lesser degree a related compound ethylenedibromide (EDB) (Takahashi *et al.*, 1981). The recognition that exposure to pesticides may be significant for workers in agriculture, and that possibly a relation exists that effects on human reproduction is more recent (Baker *et al.*, 1990). Only a few epidemiological studies refer to reproductive effects of pesticides currently used in this industry. In a study in floriculture (Restrepo *et al.*, 1990), the fungicide captan was used as a marker of exposure in a study on the prevalence of adverse reproductive outcomes in a population exposed to pesticides. Wyrobek *et al.* (1981) found that the insecticide carbaryl affected spermatogenesis of exposed production workers. The evidence, however, can not be seen as conclusive, as the proportion of abnormal sperm cells was inversely related to the duration of exposure.

In animal studies carbaryl as well as benomyl (WHO, 1986), maneb, zineb, and thiram (WHO, 1988) reduced the reproductive capacity and caused histopathological changes in the gonads. These pesticides are frequently used in fruit growing. For many other pesticides, no information is available on possible reproductive effects.

The lack of data about complex exposures in agriculture and possible inconsistencies between animal studies and human epidemiological data gave rise to an explorative epidemiological study. In this study the relation between time to pregnancy of the wives of fruit growers and occupational exposure to pesticides of the man is investigated. The fruit growing industry was selected, because this study could be incorporated into an ongoing exposure study.

MATERIALS AND METHODS

Study population

The study population consisted of 447 fruit growers out of around 3000 members of the Dutch National Fruit Growers' Organization, who participated in a research program on exposure to pesticides and were willing to participate in follow up studies. In the first phase a postal questionnaire was sent to all fruit growers to gather basic information about the population, the farms, tasks performed, and use of pesticides.

Out of this population a selection was made of all the couples with children of 12 years of age or younger. Thus, the data concern 91 pregnancies occurring between 1978 and 1990. This period was chosen, as a compromise between limited recall time and large number of pregnancies. Existing data suggest that a recall period up to about 10 years does not seriously affect the reliability of the time-to-pregnancy data (Joffe, 1989).

Exclusion criteria were the occurrence of pregnancies despite contraceptive use, no contraceptive use preceding the intention to become pregnant, and time to pregnancy exceeding 24 months (Baird *et al.*, 1985). Thereby, all data on time to pregnancies are assumed to be comparable with respect to the explicit intention of all couples to become pregnant.

Data collection

Wives of fruit growers were personally interviewed on time taken to conceive in the period of 1978-1990 by two female researchers and two female research assistants. The questionnaire was based on the questionnaire used by Baird and colleagues. A pretest of this questionnaire among eight farmer's wives, led us to delete a question about frequency of coitus, because it was experienced as inconvenient by the interviewee. Moreover, the validity of this question was expected to be low, because it is known that frequency of coitus in the past is measured with low precision and answers are subject to social expectations (Hornsby *et al.*, 1989).

Time to pregnancy was asked for by a direct question, and calculated from the date of birth, the length of gestation, and the date at which contraceptive use stopped. In the analyses the direct question on time to pregnancy was used. The questions were asked backward in time, starting with the most recent event, *i.e.* date of birth, and the most recent pregnancy.

Information was obtained on possible confounding factors such as age, contraceptive method, nursing preceding the pregnancy in question, smoking habits, alcohol consumption of the husband and consultation with a physician for fertility problems.

To ascertain possible exposure of the wife of the fruit grower, information was obtained about farm work and occupation. Information on farm characteristics and use of pesticides in 1989 was available from the postal questionnaire.

The participating farms were sent an additional questionnaire to collect information on changes that had occurred in working conditions during the 1975-1990 period in type of fruit grown, farm size, spraying equipment used and time spent applying pesticides by the fruit grower himself. These data roughly reflect the working conditions, which indicate exposure of the male to pesticides.

From a separate field study of exposure in the same population, data were available on dermal and respiratory exposure during application of pesticides. From May-October 1990, exposure to four commonly used fungicides has been measured on 149 fruit farms from the initial study population of 447 fruit growers.

Data on the most frequently used fungicide captan have been used as a marker, to study factors influencing exposure during application. Dermal exposure was measured with circular skin pads made of α-cellulose (diameter 2.5 cm), placed on the back of the neck, forehead, arm, and both wrists. Respiratory exposure of inspirable pesticide particles was measured by personal air sampling in the breathing zone (Smid *et al.*, 1992) on a glassfibre filter (diameter 2.5 cm), connected to a portable pump device with a flow of 2 L/min.

The analysis of captan was performed with high performance liquid chromatography (HPLC) with a c18 reversed phase column, connected to an UV absorbence detector (wavelength 210 nm). A mixture of acetonitrile (55%) and water (45%) was used as eluent (unpublished data).

STATISTICAL ANALYSIS

All analyses were performed with statistical analysis system software (SAS). Survival analysis was used to study the relation between exposure of the fruit grower and time to pregnancy of the couple. In univariate analyses of time to pregnancy and the independent variables, Kaplan-Meier curves were calculated with Proc LIFETEST. For multivariate analysis, the Cox proportional hazards model was used (Cox, 1972). In the PHREG SAS procedure (SAS, 1991), the fecundability ratio is calculated as e^{β}, representing the fecundability of the exposed group relative to the referent group. To account for the occurrence of ties, because time to pregnancy is expressed in months, exact maximum likelihood estimates were calculated.

Survival curves and fecundability ratios were calculated for potential confounding factors. The strength of the relation with time to pregnancy was used as criterion to add the variables in a multivariate proportional hazards model (Dales *et al.*, 1978). A physician's visit for fertility problems is considered to be a confounding factor (Baird *et al.*, 1986), because the more highly exposed couples might tend to visit a physician more often and consultation may influence fecundability through altered sexual behaviour or medication. This factor may also be considered to be an effect variable. Therefore, analyses both adjusted and unadjusted for this variable were carried out. The effect of single exposure variables was estimated, uncorrected and corrected for the set of potential confounding variables. Ordinal and interval variables were included in the analysis as dichotomous variables, with categories divided at the median to increase statistical power. Exposure variables of the man that had a significant relation to time to pregnancy and had no or low mutual correlation were considered in one multivariate model.

RESULTS

Population

From the selected group of 91 couples, 59 were willing to participate and have been interviewed. After inspection of the data, 10 couples from the initial selected group did not meet the criteria for participation, as their children were either over 12 years of age or had been adopted. For 10 non-responding couples, the subject of fertility was the main reason not to participate. The other non-respondents mentioned motivations beyond the study subject, such as pressure of work. Together, the remaining 59 couples had 133 pregnancies during the 1978-1990 period. A reliable time to pregnancy could not be calculated in all cases. The following pregnancies have been excluded: 12 pregnancies in spite of contraceptive use at the time of conception, 28 pregnancies from women who did not use any contraceptive method and one pregnancy that took over 24 months to conceive.

The final analysis includes 91 pregnancies of 43 couples. Table 1 presents relevant characteristics of the pregnancies. In 65% of the pregnancies women conceived within three months. The most frequently used contraceptives were oral contraceptives (53%) and condoms (29%), roughly reflecting Dutch contraceptive use. In 61% of the cases the data concerned the first or second pregnancy.

Analysis

Of the potential confounding variables, gravidity, expressed as the number of pregnancies (fecundability ratio 1.24, 95% CI 1.04-1.47) and visit to a physician for fertility problems (fecundability ratio 0.36, 95% CI 0.20-0.66) showed a strong relation with time to pregnancy. We found no significant relation for the use of an intrauterine device and oral contraceptives with time to pregnancy. A high gravidity is associated with a shorter time to pregnancy, which is reflected by a fecundability ratio of 1.24. Consultation of a physician is associated with an extended time to pregnancy.

In Table 2 survival analyses are presented and show a significant relation for several farm characteristics with time to pregnancy. Results presented are unadjusted, as well as adjusted for confounding variables. The use of a modern "cross-current" airblast sprayer is related to a shorter time to pregnancy. A low spraying velocity, expressed as the crop area sprayed/h (≤ 1.5 hectares/h), application of pesticides carried out solely by the farm owner, a low number of spraying days/y and a small crop area are related to a longer time to pregnancy. In a multivariate analysis with spraying velocity and application solely by the owner showed a fecundability ratio of 0.47 and 0.46 ($P<0.01$) respectively, and the significance level increased compared with the univariate analyses (Table 3). This suggests that both of these variables are independent determinants of exposure. No additional exposure variables were put into the model because of multicollinearity.

Table 1 Characteristics of study population (91 pregnancies)

Characteristic	No.	(%)
Time to pregnancy (months)		
1	32	35
2- 3	27	30
4- 6	20	22
7-12	9	10
≥ 13	3	3
Age of woman (years)		
20-24	19	21
25-29	47	52
30-34	20	22
> 34	5	5
Contraceptive use:		
Oral contraceptive	48	53
Condom	26	29
Timing of intercourse	9	10
Intra uterine device	4	4
No use of contraceptive[A]	4	4
Gravidity (number of pregnancies)		
1	25	28
2	30	33
3	23	25
≥ 4	13	14
Recent nursing, preceding pregnancy	9	10
Physician visit for fertility problems[B]	15	16
Current smokers	42	46

[A] Time to pregnancy started after birth of preceding pregnancy
[B] Woman's visit to a physician preceding most recent, or earlier pregnancy.

Table 2 Survival analyses of 91 pregnancies with one exposure variable unadjusted and after adjustment for consultation and gravidity

Variable	Fecundability ratio	(95% CI)	*P*-value
Unadjusted			
Cross current airblast sprayer	1.73	(1.10-2.72)	0.02
Spraying velocity ≤ 1.5 ha/h	0.49	(0.31-0.78)	< 0.01
Application solely by owner	0.64	(0.40-1.02)	0.06
Crop area ≤ 10 hectares	0.61	(0.40-0.93)	0.02
Spraying days ≤ 15 days/y	0.44	(0.24-0.83)	0.01
Adjusted for consultation and gravidity			
Cross current airblast sprayer	1.48	(0.94-2.34)	0.09
Spraying velocity ≤ 1.5 ha/h	0.53	(0.33-0.85)	< 0.01
Application solely by owner	0.55	(0.34-0.88)	0.01
Crop area ≤ 10 ha	0.59	(0.38-0.90)	0.02
Spraying days ≤ 15 days/y	0.48	(0.25-0.91)	0.02

Table 3 Multivariate survival analysis of 91 pregnancies for spraying velocity and application solely by the farmer

Variable	Fecundability ratio	(95% CI)	*P*-value
Consultation	0.39	(0.21-0.71)	< 0.01
Gravidity	1.38	(1.11-1.69)	< 0.01
Spraying velocity	0.47	(0.29-0.76)	< 0.01
Application solely by owner	0.46	(0.28-0.77)	< 0.01

Table 4 Farm characteristics of each exposure group (n = 43 farms)

Farm characteristic	High exposure	Low exposure
Spraying velocity (hectares/h)	≤ 1.5	> 1.5
No of farms	19	24
Traditional growing system (%)	37	13
Modern equipment (%)	11	50
Knapsack sprayer (%)	37	13
Cabin on tractor (%)	32	71
Application solely by owner (%)	63	71
Mean (SD):		
Spraying velocity (hectares/h)	1.0 (0.4)	2.5 (1.6)
Crop area (hectares)	7.9 (6.9)	12.4 (4.0)
Spraying days (1975)	28 (18)	38 (26)
Spraying days (1990)	38 (26)	39 (17)

SD = standard deviation.

In Table 4 the population is divided into categories of spraying velocity. A low spraying velocity represents less frequent use of the modern spraying technique, more frequent use of engine driven knapsack sprayers, less use of tractor cabins, a longer spraying time for each spraying and a small crop area. There is a striking increase in number of spraying days in the 1975-1990 period in the low compared with the high spraying velocity group where this number was stable. Because of the strong relationships of spraying velocity with these variables, a low spraying velocity is interpreted as relatively intensive contact to pesticides (high exposure group) and a high spraying velocity as relatively less intensive contact (low exposure group).

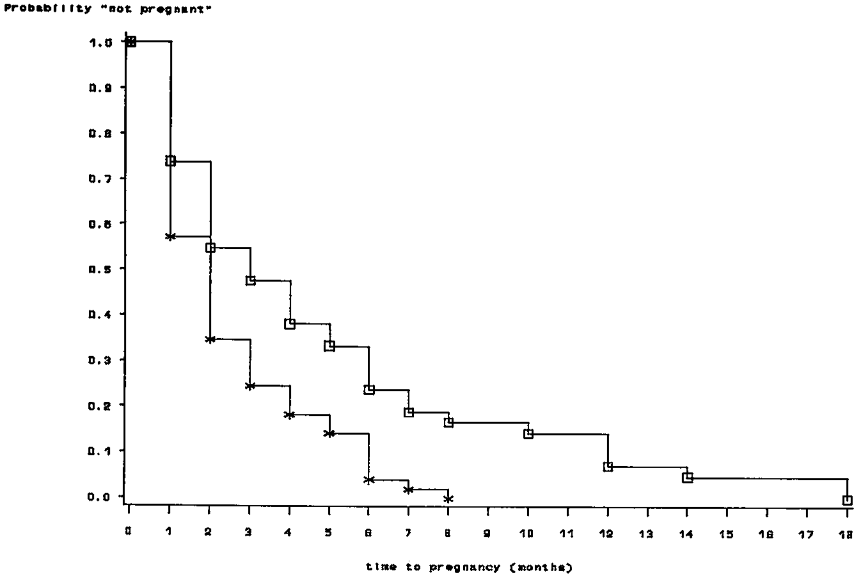
Some of the underlying variables of spraying velocity were studied in a separate exposure study. The effect of a cabin on dermal exposure was confirmed by exposure measurements during applications of captan. In Table 5, the effect of a tractor cabin and the use of an "old type" of airblast sprayer on exposure is compared with the use of a modern cross-current airblast sprayer. No differences in respiratory exposure were found for cabin use and type of airblast sprayer. Significantly lower dermal exposure of the forehead and back of the neck and of the wrists and arm was found among users of modern equipment and users of a cabin. The magnitude of the difference in dermal exposure found varied from three to 14. Exposure data were log normally distributed, therefore, the geometric SD is given. Large geometric SDs (Table 5) are not unusual for occupational exposure measurements (Rappaport, 1991). Figure 1 shows the crude effect of exposure on time to pregnancy.

As application of pesticides is a seasonal activity, the period of the year when the couple tried to conceive was included in the analysis. The spraying season is defined as the period of March up to and including November. The population is broken down into four categories according to exposure level and season. Figure 2 shows the survival curve for each category. Highly exposed farmers, who tried to conceive during the spraying season, show a time to pregnancy twice as long as the other categories. Only highly exposed farmers who tried to conceive during the spraying season (33 pregnancies) show a significantly decreased fecundability ratio (0.42, 95% CI 0.20-0.92). Out of the spraying season the effect of a high exposure was absent (fecundability ratio 0.82, 95% CI 0.33-2.02).

Table 5 Differences in dermal and respiratory exposure for different spraying equipment

	No cabin on tractor			Cabin on tractor				Old type airblast sprayer, without a cabin			Modern cross current airblast sprayer with a cabin			
	n	AM	GSD	n	AM	GSD	*P*-value	n	AM	GSD	n	AM	GSD	*P*-value
Respiratory exposure (mg m^{-3})	23	0.06	4.1	23	0.10	7.2	0.60	16	0.06	3.4	9	0.11	6.4	0.85
Dermal exposure, forehead + neck (mg m^{-2} h^{-1})	17	7.06	2.9	10	0.50	1.9	< 0.01	11	7.35	3.4	4	0.57	1.9	0.01
Dermal exposure, wrists + arm (mg m^{-2} h^{-1})	22	16.14	3.2	18	4.08	3.6	< 0.01	15	11.82	3.1	7	3.98	3.7	0.01

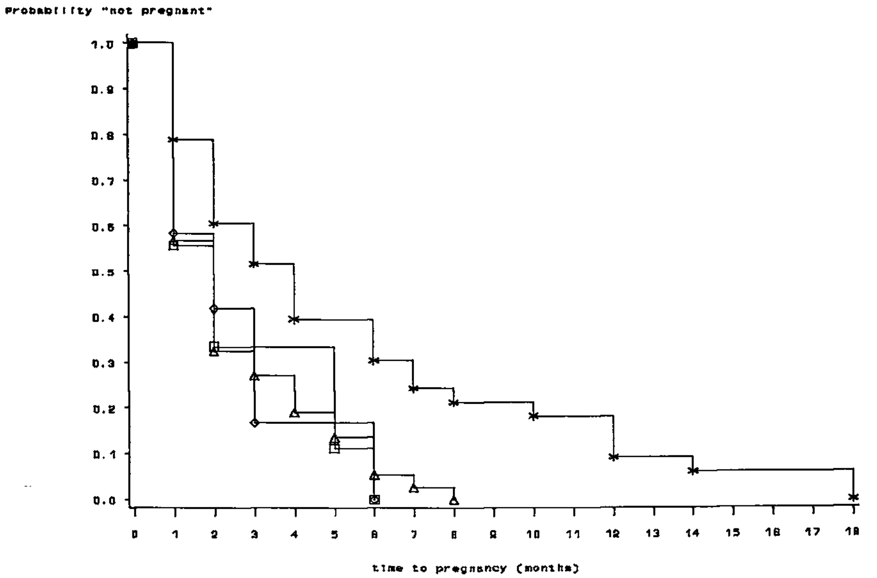
The exposure data are log normally distributed. AM = arithmetic mean; GSD = geometric standard deviation; *P*-value = *t* test on log transformed data.



□ : high exposure; spraying velocity ≤ 1.5 hectare/hour; n = 42.

* : low exposure; spraying velocity > 1.5 hectare/hour; n = 49.

FIGURE 1 Kaplan-Meier curve of time to pregnancy (n = 91) by exposure category



□ : high exposure, December - February (n = 9)

◇ : low exposure, December - February (n = 12)

* : high exposure, March - November (n = 33)

▵ : low exposure, March - November (n = 37)

FIGURE 2 Kaplan-Meier curve of time to pregnancy (n = 91) for exposure category and time of year that couple intended to become pregnant

Thus far, a visit to a physician for fertility problems was considered to be a confounding factor in the analysis. It could also be regarded as a possible effect of exposure, and may therefore have resulted in overcorrection in the previous analyses. In the high exposure group 28% of the pregnancies were preceded by a visit to a physician for fertility problems compared to 8% in the low exposure group. An analysis without this confounding variable resulted in a stronger association between the exposure variables and time to pregnancy. The fecundability ratio for spraying velocity decreased from 0.47 (95% CI 0.29-0.76) to 0.42 (95% CI 0.26-0.67).

All pregnancies have been assumed to be independent: however, theoretically pregnancies from the same couple are dependent, *i.e.* women with a long time to pregnancy might have a long time to pregnancy for all subsequent pregnancies as well. Thus, a subset of the first pregnancies (n = 25) of every couple was analysed. A similar negative effect of exposure on fecundability was shown. The fecundability ratio was 0.33 (95% CI 0.12-0.93). Because the gravidity of these pregnancies was equal to one, this confounding variable was not included in this analysis.

DISCUSSION

Crude analyses showed that the following indices of exposure were related to differences in time to pregnancy: the type of sprayer used (old or modern), spraying velocity, application solely by the farm owner, crop area, and frequency of sprayings a year. After adjustment for confounders in a multivariate analysis the strongest relation remained with the application of pesticides by the owner of the farm and spraying velocity. These two variables seemed to be independent indicators of exposure. Application of pesticides solely by the farm owner most probably leads to a longer duration of contact with pesticides each season, compared with farmers who share this task with other individuals. Spraying velocity is assumed to be a good indicator of intensity of exposure, because it probably leads to a more intensive contact with pesticides. Working conditions, such as the use of less modern equipment associated with a low spraying velocity, result in a higher exposure. A low spraying velocity mainly occurred on small farms. This category showed an increase in number of spraying days in the 1975-1990 period, without keeping step with the use of modern equipment. A higher spraying velocity, however, which may result in a lower exposure, does not necessarily imply a lower overall use of pesticides. These farms generally have a larger crop area and a higher crop density, and thus consume larger amounts of pesticides. It is, however, reasonable to assume a lower personal exposure here, due to the differences in working conditions during the application of pesticides.

It is unlikely that qualitative differences in pesticide exposure exist within this population, for instance because different pesticides are used. On all farms the main crops grown were apples and pears, and fruit growers generally rely on the annual publication by the Dutch National Fruit Growers' Organization of recommendations on pesticide use. These detailed recommendations comprise the use of specific pesticides, dosage, and frequency of use for each crop.

The exposure variables such as application of pesticides by the farm owner and spraying velocity are directly related to the exposure of the applicator. Usually mixing, loading, and applying of pesticides is a task exclusively of the men. The women often participate in tasks involved with the crop such as bending, pruning, thinning and harvesting, which may result in exposure as well. Also contact with contaminated clothing or contamination of the home may be a source of exposure. Thus, it cannot be ruled out that part of the effect is mediated by exposure of the wife. The seasonal effect found supports the relation between intensity of exposure to pesticides and fecundability. A seasonal effect also suggests that the decrease in fecundability is reversible.

Attempts were made to validate the qualitative indices of exposure. It could be shown that some of the underlying factors that determine spraying velocity like the use of a tractor cabin or use of an old airblast sprayer were related to a higher dermal exposure. Exposure measurements during the application of the fungicide captan showed that use of a cabin on the tractor and use of modern equipment indeed resulted in a lower dermal exposure but no difference in respiratory exposure. This supports the validity of the exposure indices used. The exposure data cannot be interpreted as quantitative measure of exposure dose, because exposure to pesticides was restricted to application of only one pesticide. It was impossible to validate the variable spraying velocity, because spraying velocity also represents other application techniques such as use of a knapsack sprayer and the frequency of spraying days each year, for which no exposure measurements were available. Furthermore, other tasks for which no exposure measurements were available, like pruning and thinning may add greatly to the total exposure dose of farm workers. It is important to note that captan was used here as a marker of exposure to pesticides in general. In a study of Restrepo and coworkers, captan was also used as a marker of exposure in a study on the prevalence of adverse reproductive outcomes in a population exposed to pesticides (Restrepo *et al.*, 1990). In their study only respiratory exposure was taken into account. A striking difference is that the exposure data in our study suggest that the dermal route may play a predominant part in determining exposure dose. On the other hand it is important to realise that to use one component as a marker of exposure for a large variety of different pesticides, which may result in mixed exposures, is beset with problems. The importance of the exposure route depends on the physical and chemical properties of a chemical. Also, respiratory exposure normally stops directly after the job has been finished, whereas dermal uptake may continue, depending on the hygienic behaviour of the worker. And, moreover, exposure of the skin will not be uniformly distributed over the body surface (WHO, 1990).

Alternative explanations

A differentiation in spraying velocity and related factors such as farm size, possibly runs parallel with socioeconomic status, which could be a confounding variable (Baird *et al.*, 1986). The chance that this could have an effect in the same order of magnitude as the effect of spraying velocity is not plausible, especially because fruit growers form a relatively homogeneous socio-economic population.

Another possible explanation is a higher degree of participation of the wife in farm work resulting in exposure when in the orchard. For both exposure categories the type of work performed by the wives is the same. On the larger farms more work is to be done, but generally more workers are hired. Unfortunately, no information is available on time spent on the farm by the wives, so a definite answer cannot be given. A direct relation to pesticide exposure of the man is more plausible, although a combination of both is still possible.

Finally, it is unlikely that the increased time to pregnancy of couples trying to conceive during the spraying season is due to indirect effects such as high work load, stress, or a decreased frequency of sexual intercourse, because time to pregnancy was not increased in the farmers with a low exposure.

Quality of data

Both the exposure and effect variables are retrospective, self reported data. We assume the validity of the fecundability data to be high because the respondents were generally convinced by their own answers. A high validity of fecundability data from face to face interviews is supported by a study of Zielhuis *et al.* (1992).

Another supporting argument is the coherence of the expected relations between time to pregnancy and confounding factors. For instance the relationship between time to pregnancy and gravidity (Weinberg *et al.*, 1989), and a visit to a physician for fertility problems (Baird *et al.*, 1986). A lower fecundability was also found for older women and for recent nursing, but because of the small numbers, these variables were not taken into account in the analyses. An important explanation for the absence of an effect of smoking and alcohol consumption is probably the absence of extremely heavy smokers and drinkers (Baird *et al.*, 1985).

The exposure data apply to the 1989 situation, whereas pregnancies concern the 1978-1990 period. In general, information from publications for crop protection and spraying calendars shows a stable use of pesticides, but changes in working conditions have occurred during the 1975-1990 period. The number of tractor cabins has been increased and the cross current airblast sprayer was introduced in 1982. Possible misclassification of older pregnancies will have resulted in a dilution of the observed effect.

Moreover, the use of crude exposure variables can only mask the effect by non-differential misclassification (Ahlbohm *et al.*, 1990). Differential misclassification is not probable because data on exposure and time to pregnancy were collected independently. Both the respondent and the interviewer were uninformed about actual exposure levels.

Selection bias

Non-respondents who were not willing to participate form a potential source of bias. Underlying motivations could have been objection because of personal convictions (taboo, intimacy) or personal experiences with reproductive problems. The couples with reproductive problems (n = 10) form a systematic source of bias, dependent on the exposure resulting in either a stronger or weaker effect. Given the strong relation and the small number of non-respondents, it does not seem plausible that this bias will affect the conclusions.

Another important potential source of bias may be caused inherently by the method of calculating time to pregnancy. This excludes infertility, which may cause a serious underestimation of the effects of pesticide exposure on reproduction.

Concluding remarks

The results indicate that a negative effect of exposure to pesticides on fecundability is present. The exposure variables used, globally indicate the real personal exposure dose. It is impossible to draw conclusions about specific pesticides responsible for the effect, or about the underlying mechanism. Further research in fruit growing is concentrating on the measurements of quality and quantity of exposure to pesticides, not only during application of pesticides, but also during other tasks performed in the orchards.

Probably, the role of the skin as an exposure route of pesticides is underestimated. Because of lack of knowledge concerning the uptake of chemicals through human skin, a reliable estimation of the internal dose is hardly possible. Besides, in contrast with respiratory exposure, validated methods to measure dermal exposure are not yet available.

Although the threshold limit value TLV for captan of 5 mg/m^3 for the air borne concentration (ACGIH, 1990) has not been exceeded for fruit growers, one can doubt if TLV's for pesticide exposure through the respiratory route only (when available) will safeguard occupationally exposed workers in agriculture.

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CHAPTER 7

Offspring sex ratio as an indicator of reproductive hazards associated with pesticides

ABSTRACT

The hypothesis that human offspring sex ratio (male/female ratio) might be affected by environmental or occupational factors is not new, but the evidence is weak. The underlying mechanisms have not yet been elucidated. Effects on chromosome level, resulting in death in utero for male has been suggested. Other evidence points to a possible influence of hormone levels of the parents at the time of conception. Several larger studies on deviations in sex ratio have shown inconsistent results. Some other studies were hampered by small study populations, poor estimation of individual exposure or poor control for the many potential confounders. Some weak scientific evidence states that exposure to pesticides might affect offspring sex ratio. In 1987 an excess of daughters among the offspring of a small group of male 1,2-dibromo-3 chloropropane (DBCP) applicators was observed (Potashnik *et al.*, 1987). An advantage of offspring sex ratio as a marker for possible health effects is that it is in principle unbiased and easy to monitor through vital records. If sensitive, this measure may serve as a warning sign for potential adverse effects of environmental or occupational exposures.

In reply to a letter to the editor (James, 1995) on our study on time to pregnancy (de Cock *et al.*, 1994), offspring sex ratio was studied in the same population. From our results among fruit growers we concluded that it seems useful to explore further if sex

ratio is linked to pesticide exposure and if both sex ratio and time to pregnancy are interlinked.

INTRODUCTION

Human offspring sex ratio, expressed as the male/female ratio, generally is somewhat higher than one. Differences in this ratio, however, exist between countries (Williams 1992) and also unexplained significant temporary variation have occurred (McDowall, 1985). The sex ratio of offspring is a complex matter, especially in reptiles and invertebrates. (Karlin *et al.* 1986). The progeny of invertebrates range from all male to all female, depending on ambience. The high incidence of a 1:1 sex ratio at birth in mammal populations is striking as stated by Karlin, compared to the frequent deviations from this ratio in invertebrate species. An approximate 1:1 sex ratio appears to predominate in most vertebrates in the early stages of development.

In The Netherlands the overall human offspring sex ratio between 1900 and 1993 ranged from 1.035-1.077 and was on average 1.056 (CBS, 1994). Based on these data there seems to be a slight decline in sex ratio since 1945 (Figure 1). In 1994 the overall sex ratio in The Netherlands was 1.050 (CBS, 1986, 1994).

Variation in human sex ratio within a population, has been attributed to genetic (Beck, 1992) and disease factors, but also to environmental factors such as air pollution (Williams, 1992 and 1995), and specific occupational exposures (Röckert 1977, Lyster 1982, McDowall 1982, 1985, Potashnik 1987, Larsen 1991, Milham 1993, James, 1994, and Whorton 1994). Examples of extreme, statistically significant, shifts in offspring sex ratio were a sex ratio[1] of 0.83 (proportion of boys) in men treated with gonadothropin (James, 1989), a sex ratio of 0.33 in men exposed to the pesticide 1,2-dibromo-3 chloropropane (DBCP) (Potashnik *et al.* 1987) and a sex ratio of 0.33 in non-hodgkin's lymphoma patients (Olsson *et al.*, 1982).

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[1] Sex ratio will be defined throughout the text as proportion of boys if not specified otherwise

Mechanisms

The underlying mechanisms of variation in offspring sex ratio have not yet been elucidated. The theory behind changes in sex ratio mentioned by Buffler *et al.* (1982) is that certain recessive mutations of genes of the X-chromosome will not produce an effect in female, but may effect the male (with only one X-chromosome) and may result in death in utero. In their study on reproductive outcomes in families of men exposed to the pesticide 1,2-dibromo-3 chloropropane (DBCP), Goldsmith *et al.* (1984) mentioned Y chromosome non-disjunction as a possible explanation. Other evidence, points to a possible influence of hormone levels of the parents on sex ratio (James 1989, 1990, 1992). It was suggested that a high level of testosterone and oestrogen favour the production of males. On the other hand, high levels of gonadotrophin and progesterone favour females (James 1992). However, the underlying mechanisms of a hormonal influence have not been specified.

Environmental studies

A geographical analysis showed locations with statistically significant excesses of female births in areas at risk from airborne pollution from incinerators in Scotland (Williams *et al.* 1992). The authors hypothesized that, although the mechanism of how pollution could affect the sex ratio of birth is not clear, the metabolism of the rapidly dividing cells of the gonadal and fetal tissues is likely to be particularly vulnerable to the influence of pollutants. Williams (1995) cited other retrospective studies which showed abnormal sex ratios in residential areas exposed to air pollution. A possible role on respiratory cancer and high sex ratio's of births was suggested of air pollution in a small industrial town in Scotland (Lloyd 1984). These findings were replicated in another town and the surrounding area of that town (Lloyd 1985). Also, the London smog during five days in December 1952 has been studied in this respect. Births at London hospitals had a sex ratio of 75 males per 100 females, 320 days after the exposure (Lyster, 1977).

Williams *et al.* (1995) carried out another study on air pollution, sex ratio, and mortality in localities where pollution was present from a broad range of industrial sources and probably in lower concentrations or of a less specific nature. The authors concluded that sex ratios were not consistently affected when the concentrations or components of the air pollution were insufficiently toxic to cause substantially increased death rates. Therefore, it was concluded that sex ratio does not provide a reliable screening measure for detecting health hazards from industrial air pollution in the general residential environment (Williams, 1995).

In one case also the relation between drinking water contamination with DBCP in California and sex ratio was studied. It was concluded that offspring sex ratio was not affected (Whorton *et al.*, 1989).

The relationship between sex ratio and irradiation of the atomic bombs in Hiroshima and Nagasaki has been studied as well. On the whole, no evident relationship between either dose or trimester and the sex ratio was found (Jablon *et al.*, 1971). The findings did not confirm earlier findings of a higher proportion of males (67% males) as reported by Meier *et al.* (1969). That study, however, concerned a population of females exposed to x-rays for medical purposes.

Occupational studies

Several studies on sex ratio in relation to occupational exposures have been carried out. Offspring sex ratio by parents' occupation in England and Wales was studied by McDowall (1985). A first major analysis related to data of 1931 of over 600.000 births, suggested that sex ratio might be related to the enormous range of potentially dangerous substances in the occupational environment. A repeated analysis for 1978 and 1980-82 data supported some of the earlier findings. It was noted that because of multiple comparisons of many occupations in the analyses, the consistency of findings, either with previous observations or across related occupations would be a better indicator of significance. There might have been changes in occupational exposure and social factors over the years and therefore comparisons should be made with caution. The 1978 and 1980-82 data were suggested to be more appropriate than the earlier data in that respect. A low ratio for farming and underground coal miners in 1978 was not supported in the 1980-82 data. A low sex ratio was found for textile workers in both studies. Also warehousemen and UK armed forces showed similarly reduced ratios in both studies. Milham (1993) reported a highly significantly low sex ratio of birth to male carbon setters. These workers are expected to be highly exposed to heat, air pollution and strong magnetic fields. An excess of daughters was also found for offspring of deep divers in the Swedish Royal Navy (Röckert 1977) and for Australian abalone divers (Lyster, 1982). A statistically significant excess of daughters among the offspring of male DBCP applicators was observed by Potashnik *et al.* (1987).

Hormone levels are mentioned to play a role. It was suggested that in case of divers the low offspring sex ratios were due to low testosterone levels (James, 1994). This was substantiated by literature of Röckert *et al.* (1978), who reported reduced testosterone levels of rats exposed to a hyperbaric environment and a decrease in plasma testosterone in humans after diving (Röckert *et al.*, 1983). It was suggested that normal testosterone, but elevated gonadotropin levels were responsible for the observed effects among DBCP workers (James 1994). Larsen *et al.* (1991) reported that female Danish physiotherapists exposed to low levels of electromagnetic radiation produce a highly significant, dose-related excess of daughters. James (1995) suggested that high gonadotrophin levels in these women might have been responsible. However, a study of Gubéran *et al.* (1994) among 2846 female members of the Swiss Federation of Physiotherapists did not confirm this finding on sex ratio. It was also hypothesized that a significantly high proportion of females in the offspring of pilots of high performance aircrafts might be related to their lower testosterone levels (James, 1988).

Negative studies were also reported. A study among male employees of a mining and production facility exposed to sodium borates showed no adverse reproductive effects (Whorton *et al.*, 1994). Also in a study on adverse pregnancy outcome and childhood malignancy in relation to paternal welding exposure no change in sex ratio was observed (Bonde *et al.*, 1992). As low testosterone and high follicular stimulating hormone levels in welders have been reported previously, it was suggested that a study population of 2241 might have been to small to detect a possible effect in this case (James, 1994).

Summarizing the occupations with a deviated lower offspring sex ratio, which were mentioned in one or more studies, though sometimes with conflicting results were: *farming, underground coal miners, textile workers, warehousemen, UK armed forces, carbon setters, deep divers, abalone divers, DBCP applicators, physiotherapists, and pilots of high performance aircrafts.* Only the study on DBCP-applicators pointed to a specific exposure.

Effect of diseases on hormone levels

An unusual sex ratio caused by unusual hormone levels may also reflect pre-onset of a disease situation in the father (James, 1994). As some diseases of the parents are also hormonally related, it is not clear if changes in sex ratio can be attributed to an environmental or occupational factor or to a pathology of the parents. Recently, Glaser (1994) suggested for instance that sex differences in Hodgkin's disease are consistent with an involvement of reproductive factors in women, suggesting a role of hormonal factors. For some parental diseases an association with unusual offspring sex ratios has been mentioned.

A higher sex ratio was found in relation to prostatic cancer (James, 1990), and hepatitis B (Drew, 1978, Chahnazarian *et al.* 1988), and multiple sclerosis (Alperovitch *et al*, 1981, Verdier-Taillefer, 1991). For multiple sclerosis, however, a significantly lower sex ratio for male and a non-significant higher ratio for female patients also was mentioned (James, 1990).

A lower sex ratio was found for otosclerosis (James, 1989), and Non-Hodgkin's lymphoma (Olsson *et al.* 1982). It was suggested (James, 1994) that for some of these diseases the unusual sex ratio is a hormonally mediated consequence of the disease (*e.g.*, non-Hodgkin's lymphoma and multiple sclerosis), but that for instance for prostatic cancer an unusual hormone profile may be responsible for both the disease and the shifts in sex ratio.

Sex ratios of offspring conceived prior to onset of disease would reflect pre-onset hormone levels. On the other hand, it has been suggested that treatment for the disease may have an influence on sex ratio (Verdier-Taillefer, 1991). For instance treatment with steroids may be relevant (Chee D, 1995[2])

Another point in relation to hormone levels and the occupations of the parents is that testosterone is associated with many facets of personality (James, 1992). It has been associated with dominance, aggression, antisocial behaviour, sensation-seeking, persistence and libido, which may also be related to choice of occupation. Abalone diving,

[2] Personal communication, 13 June 1995, London School of Hygiene and Tropical Medicine

alcohol-related occupations, and professional drivers are occupations thought to lower testosterone levels. It is known that chronic alcoholics have lower testosterone levels. For professional drivers a relation with low offspring sex ratio was postulated based on evidence that lead poisoning depresses sperm quality, that sperm quality of professional drivers is poor, and evidence that lead lowers the testosterone level, but not the gonadotrophin level of male rats (James, 1992).

Xeno-hormones

Recently, Sharpe and Skakkebaek (1993) have suggested that the assumed increasing incidence of reproductive abnormalities in the human male in the past half-century may be related to increased exposure to xeno-oestrogens in utero (Editorial, 1995; Sharpe *et al.*, 1993). Xeno-oestrogenes are environmental chemicals with oestrogenic activity. Exposure of the male fetus to supraphysiological concentrations of oestrogen compounds is mentioned as the possible triggering factor. Oestrogenic pollutants which are held responsible for the reproductive changes in animals range from: organochlorine pesticides, to polychlorinated biphenyls, dioxins and furans, alkylphenol polyethoxylates, phyto-oestrogen's (natural compounds present in plants) and other xeno-oestrogen's such as phthalates. Some of these products may result from combustion processes. Although a global decline has been suggested in male reproductive health, there might be geographical differences. For instance in Finland there has been virtually no fall in sperm concentration and a lower incidence of testicular cancer and hypospadias was reported (Editorial, 1995).

Recently, also attention was paid to environmental androgens (Gray *et al.* 1994). The fungicide Vinclozolin was found to inhibit sexual differentiation in male rats in an antiandrogenic manner. The metabolite p.p'-DDE of DDT was suggested to have an effect on the androgen receptor as well (Kelce *et al.*, Sharpe, 1995).

Confounders

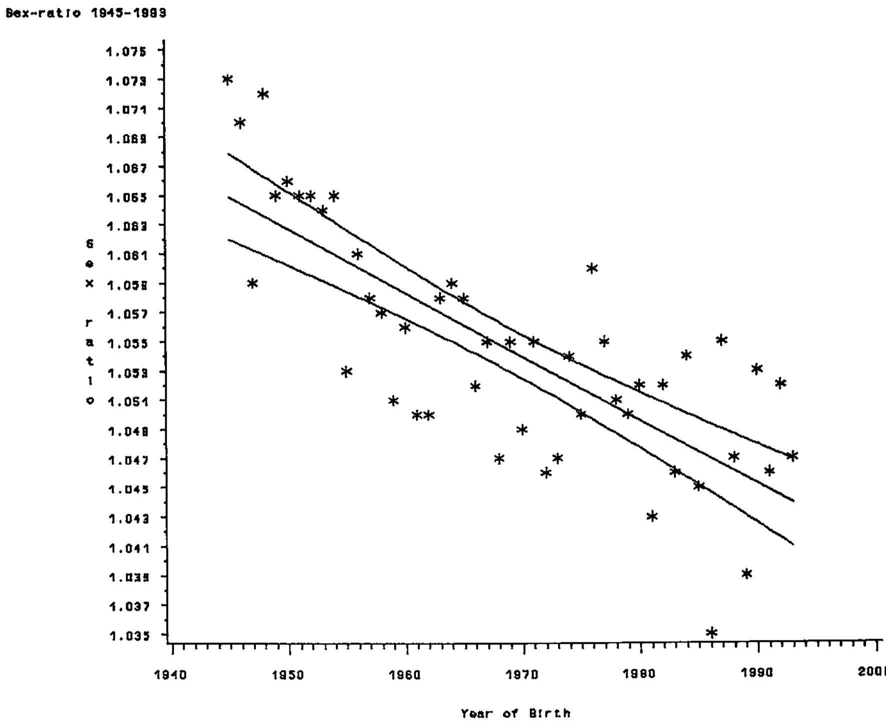
Potential confounders which may be important when studying the relation between offspring sex ratio and environmental or occupational exposures are: race, social class, parental age, parity, coital frequency, cycle day, season, pill use, hormonal treatment for subfertility, prior abortion, ectopic pregnancies/placenta previa, genetical predisposition and diseases, blood group, alcohol consumption, and smoking of females (Table 1). Not for all potential confounders, it is clear how important they might be in studies on offspring sex ratio and often the evidence is not very extensive.

Concluding remarks

The hypothesis that sex ratio might be affected by environmental or occupational factors is not new. It is, however surprising that the possible underlying mechanisms have not been studied more systematically. Several studies have shown inconsistent results and some of the studies were hampered by small study populations, poor estimation of individual exposure or poor control for the many potential confounders.

To test the hypothesis of a possible influence of hormone levels, future research should combine the measurement of exposure with measuring of hormone levels.

There are advantages in using offspring sex ratio as a marker for possible health effects. This measure is not subject to responder bias, it is a non-rare event, and is easy to monitor through vital records. An abrupt or persistent change can serve as a warning flag for further investigation (Buffler *et al.*,1982). If data are collected directly from a study population, however, the willingness to participate may depend on social acceptance (selection bias). Many questions have to be answered before one can address the causality of a relation between an environmental or occupational exposure and a shift in offspring sex ratio. Important in this respect are the influence of confounders and the underlying mechanisms in relation to the exposures under consideration.



Period 1945 - 1993[3]; Linear regression: n = 49, regression coefficient -4.38 10^{-4}, standard error (SE) 5.28 10^{-5}, explained variance (R^2) 0.59, P=0.0001.

Figure 1 Decline in offspring sex ratio in The Netherlands between 1945 - 1993 (CBS, 1994)

[3] Period 1900 - 1993
Lineair regression: n = 66, regression coefficient -2.17 10^{-4}, SE 3.85 10^{-5}, R^2 0.33, P=0.0001

Table 1 Potential confounders studying the relation between offspring sex ratio and environmental or occupational exposures

Confounder	Sex Ratio	References
1. race	↓ non-white *vs* white	[1,2]
2. social class	↑ upper class	[3]
3. parental age	-	[4]
4. parity	↓	[5,6]
5. coital frequency	↑	[7,8,9]
6. cycle day	U-shape	[10,11]
7. season	↑ early summer, ↓ late fall/winter	[3,7]
8. contraceptive use (pill)	-	[3,12,13]
9. hormonal treatment for subfertility	↓	[3]
10. prior abortion		[3]
- spontaneous	-	
- induced	↓	
11. ectopic pregnancies		[3]
- placenta previa	↑	
- extrauterine pregnancy	↓	
12. genetical predisposition and parental diseases		
- prostatic cancer, multiple sclerosis, hepatitis B	↑	[1,14,15,16]
- multiple sclerosis, otosclerosis, non-hodgkin (pre)-eclampsia	↓	[1,17,18,19]
13. blood groups	?	[3]
14. alcohol consumption	↓	[20,21]
15. smoking of females	↓	[3]

References: [1] James (1990); [2] Ross *et al.* (1986); [3] James (1987); [4] Martin *et al.* (1992); [5] Tarver *et al.* (1968); [6] CBS (1986); [7] James (1971); [8] James (1989); [9] James (1994); [10] Guerrero, 1974; [11] Harlap (1979); [12] James (1973); [13] Shiono *et al.* (1982); [14] Alperovitch *et al.* (1981); [15] Drew (1978) [16] Chahnazarian *et al.* (1988); [17] Verdier-Taillefer (1991);[18] Olsson (1982); [19] Arngrimsson *et al.* (1993); [20] James (1992); [21] Dickinson *et al.* (1994).

Reply to a letter to the editor

An explorative study among fruit growers in The Netherlands showed that an adverse effect of exposure to pesticides on fecundability from time to pregnancy data is likely (de Cock *et al.* 1994). In reply to a letter to the editor on the fecundability study (James, 1995), it was considered that also sex ratio might be a useful measure to study in relation to pesticide exposure. We hypothesized that there might be a difference in time to pregnancy with respect to sex. Although our study population has the drawback that the study population was small, we hypothesized that considering both sex ratio and time to pregnancy simultaneously may have advantages in studies on occupational hazards of pesticide exposure. Additionally, a variable such as families with predominantly daughters or sons, which show a shift in sex ratio, might be powerful in this respect. It seems useful to explore this further, since little is known up to date about the underlying mechanisms of exposure to pesticides on reproduction or a possible interrelation between both effect measures. (de Cock *et al.*, 1995).

Offspring sex ratio as an indicator of reproductive hazards associated with pesticides[4]

Editor——We would like to thank James for his letter on offspring sex ratio among children of fruit growers in our study on time to pregnancy[1]. In his letter James refers to a highly significant and large excess of daughters among the offspring of male 1,2-dibromo-3 chloropropane (DBCP) applicators[2] and wonders if information on sex ratio is also available for the fruit growers in our study. In our initial survey, we did not gather these data. As data on sex ratio are easy to obtain, we gathered this information recently by telephone. We asked wives of fruit growers the outcomes of their pregnancies. Except for one pregnancy, we were able to gather all the information concerning 140 pregnancies. The total number of pregnancies was 127 (excluding 12 miscarriages for which sex ratio was unknown and one pregnancy before the period of study). The overall sex ratio was 0.51 with a 95% confidence interval (95% CI) of 0.43-0.59[5]. For the 91 pregnancies in our time to pregnancy study, the overall sex ratio was 0.51.

In a more detailed analysis we first related sex ratio to the exposure variables that were also studied in the time to pregnancy study. A decrease in sex ratio was found when

[4] Wageningen Agricultural University, Department of Epidemiology and Public Health: Johan de Cock, Dick Heederik, Erik Tielemans; University Hospital Utrecht, Department of Obstetrics and Gynaecology, Section Fertility: Egbert te Velde, Roel van Kooij. Occupational and Environmental Medicine 1995;52:429-430.

[5] Based on a binomial distribution with expected population value for sex ratio (proportion of males) of 0.514[3].

recent years of birth were compared with earlier pregnancies. Also, time to pregnancy increased with more recent years of birth. (Table 1).

Table 1 Offspring sex ratio and time to pregnancy according to year of birth (n=85)*

Year of birth	Pregnancies (n)	Sex ratio	Time to pregnancy (months)
1978-80	18	0.56	2.9
1981-83	22	0.55	3.5
1984-86	24	0.58	4.2
1987-90	21	0.33	4.1

* N=91 pregnancies from the time to pregnancy study, excluding six miscarriages of unknown sex.

The most recent period (1987-90) showed a lower sex ratio of borderline significance (0.33) compared with the previous periods (0.56) (Fisher's exact test, two sided, $P=0.08$). A similar trend in sex ratio was found for the total group of 127 pregnancies. We also found a change in sex ratio dependent on gravidity. For the first, second, third and subsequent children, sex ratios of 0.60, 0.57, 0.42, and 0.31 were found respectively. The first two pregnancies of a couple in comparison with next pregnancies showed a sex ratio of 0.58 and 0.38 respectively (Fisher's exact test, two sided, $P=0.08$).

This raises the question whether gravidity acts as a confounder in this analysis. Because of small numbers, stratification of sex ratio according to gravidity and year of birth was not possible. Surprisingly, a difference in time to pregnancy according to year of birth was found for boys but not for girls. Figure 1 is a Kaplan-Meier curve (PROC LIFETEST) by year of birth for boys. The curves did not differ significantly. A univariate survival analysis with the PHREG SAS procedure as described in our study on time to pregnancy[1], for the period of birth comparing pregnancies occurring in 1983 or before (1) with more recent pregnancies (0) as the independent variable, showed a fecundability ratio of 1.61 (95% CI 0.83-3.13) for boys and 1.13 (95% CI 0.59-2.15) for girls. No differences in age at the time of conception of men or women, or the age difference between both parents were found in our study. Therefore, a role of age dependent hormone concentrations of the parents on offspring sex ratio at the time of conception is not a very likely explanation for these findings.

In our study on time to pregnancy, we focused on seasonal effects of exposure of men. No significant differences according to season were detected in the sex ratios. Observed sex ratios for the quarter of a year in which conception took place were: 0.64 (January - March), 0.44 (April - June), 0.48 (July - September), and 0.52 (October - December).

As no relation between sex ratio and any of the exposure variables used in our study on time to pregnancy was found, other available information on exposure was considered as well. Because offspring sex is a dichotomous variable, we studied outcome in a case-control like design with maximum likelihood logistic regression models by computing odds ratios (ORs) with SAS PROC LOGISTIC. As the odds ratio for gravidity (OR = 1.35, 95% CI 0.92-1.99) was not significant and adjustment did not influence the regression coefficient or standard errors of variables of interest, only crude ORs are given.

No relation was found for most of the variables studied in our time to pregnancy study, but the results suggest a relation between use of a cross current airblast sprayer (days/y) (OR=1.3, 95% CI 0.96-1.72) and use of a herbicide sprayer (days/y) (OR=2.0, 95% CI 1.08-3.76) and production of daughters. Also we compared families with more daughters than sons with the other families. Instead of gravidity we corrected for the number of children within a family (OR = 1.23, 95%CI 0.66-2.31). As some families also conceived children before the time of the study (1978-90) number of children is a surrogate measure for gravidity. Because differences with or without adjustment were small, Table 2 gives only crude ORs. For the 43 families a relation was found between the number of days with use of a herbicide sprayer (days/y), a cross current airblast sprayer (days/y), and a knapsack sprayer (days/y) and the number of daughters within a family. When comparing families with more daughters than sons (16 families), the number of spraying days a year was 37 compared with 25 for the other families, 7.6 *vs* 3.3 days/y for the herbicide sprayer, 16 *vs* 5.4 days/y for the cross current airblast sprayer, and 7.1 *vs* 3.0 days/y for the knapsack sprayer. In families with more daughters use of the specific pesticides Azinphos-methyl (insecticide), Metiram (fungicide), and Paraquat (herbicide) was higher. Table 2 shows the results of the ORs.

Table 2 Odds ratio of 16 cases (more daughters than sons within a family) compared to 27 controls (43 families) for exposure variables (See also annex 1)

Variable	Odds ratio	95%-CI
Spraying days 1990 (30 days)[A]	18.4	1.72-196
Cross current sprayer (10 days/y)	1.7	1.05-2.59
Herbicide sprayer (5 days/y)	3.6	1.10-11.6
(Manual)knapsack sprayer (5 days/y)	2.2	1.06-4.66
Metiram (1 spraying/y)	1.3	1.05-1.61
Azinphos-methyl (0/1)	4.4	1.11-17.3
Paraquat (0/1)	>5.6 *	5.6-<13.4

[A] Number of spraying days varied between 7 and 55 days in 1990
* Paraquat was used by all families with more daughters compared with 74% in the control group.

For families with more sons than daughters (17) a significant OR was found for use of a cross current sprayer (yes or no, OR = 0.16, 95% CI 0.03-0.82), and number of spraying days with this type of sprayer (OR = 0.54 per 10 spraying days/y, 95% CI 0.30-0.98).

DISCUSSION

Overall sex ratio was not different from the expected ratio of 0.51. James hypothesized that high concentrations of testosterone at the time of conception produce boys, and high concentrations of gonadotrophin produce girls. Among the offspring of DBCP applicators[3] a highly significant excess of daughters was found. As exposure to pesticides in fruit growing typically includes mixed exposure to many different compounds, similar to that found for DBCP, it is not possible to predict the direction of a shift in sex ratio induced by exposure to pesticides among this group. As the number of subgroups is small it is impossible to draw firm conclusions, but some of the results are of interest. From our results there are some indications that exposure to pesticides in agriculture may affect offspring sex ratio. The shift towards daughters in the most recent period (sex ratio of 0.33) was remarkable. Also the finding that spraying frequency, frequency of use of specific equipment, and use of some specific pesticides are related to a shift towards more daughters within a family may point to an exposure effect. One should be careful in interpreting these results. The fact that use of several pesticides is related to sex ratio does not necessarily imply that individual pesticides are causally related to sex ratio. Fruit growers use a complex mixture of agents and use of one agent is often correlated to use of another one. It is unlikely that the shift in sex ratio is caused by the introduction of particular pesticides, as all pesticides have been applied to some extent during the study period. Because application techniques changed considerably over time, the introduction of certain techniques seems a more plausible explanation for this finding. It is possible that with certain underlying mechanisms, effects may not be caused by exposure of the male worker only, as most woman live near the orchard and they often participate during particular activities like pruning, thinning and harvesting. No seasonal trends were found for time to pregnancy. Our finding that there might be a difference in time to pregnancy for boys and girls as well, may indicate that both sex ratio and time to pregnancy are interlinked. We cannot explain why exposure variables associated with time to pregnancy are not related to sex ratio.

In conclusion, we think that the suggestion by James to analyse sex ratio is a useful one and should be explored further. To consider both sex ratio and time to pregnancy simultaneously may have advantages in elucidating occupational hazards of (pesticide) exposure. Our results do show that other variables such as families with predominantly daughters or sons, which are indicative of a shift in sex ratio, might be more powerful because they use another sampling unit (family instead of a crude stratification by exposure). Especially in this study among agricultural workers an analysis on family level might be relevant because the exposure might be aggregated at the family level as well. In general, it seems useful to explore, after time to pregnancy and sex ratio, the presence of families with a predominance of one of the sexes as little is known about the underlying biological mechanisms as well as the statistical properties of these indices.

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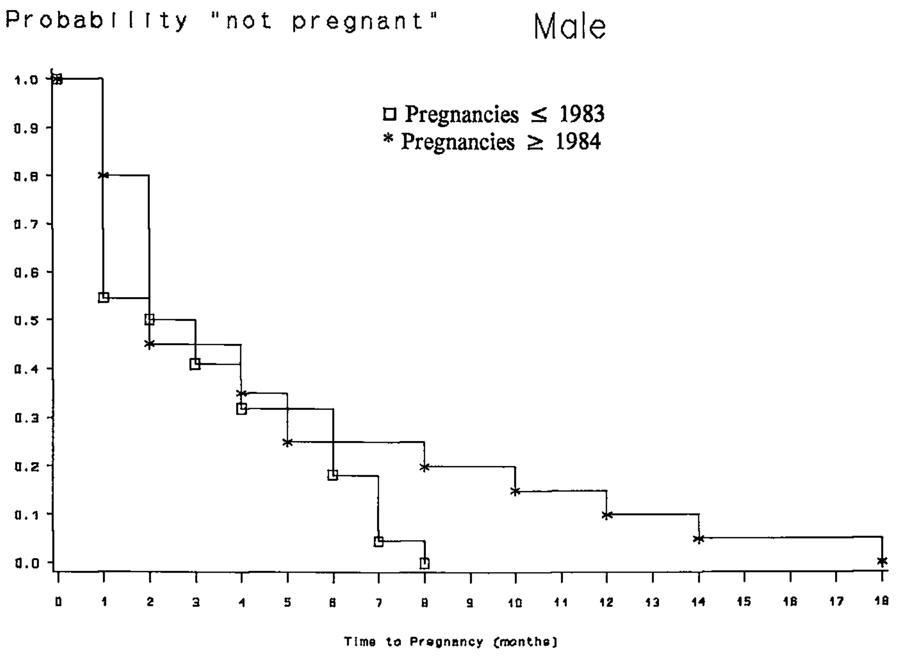

Y-axis: Probability of men not producing pregnancy

Figure 1 Time to pregnancy by year of birth for boys (n = 44)

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Annex 1. Most commonly used pesticides

Many different pesticides are used in fruit growing, but some are only used by a small percentage of farms or very infrequently. Metiram, azinphos-methyl and paraquat (Table 2 in the main text), belonged to the most frequently used pesticides. Tolylfluanid, also showed a significant difference in use between families with more daughters (median 3.4 spraying/y) and other families (median 2.2 sprayings/y) Chi square: $P=0.001$. The most commonly used pesticides on the 43 farms are given in Table A-1 and A-2. In Table A-3 and A-4 the odds ratios of the families with more daughters are given for these pesticides.

If significance tests are performed for more pesticides, the chance will become larger that some will produce statistically significant results by chance. Often the Bonferroni inequality method is used to correct for this multiple comparisons problem (Jones DR *et al.*, 1982). The significance level suggested for each statement, if the family level is to be conventional 0.05, is 0.05/n, where n is the number of statements.

The issue of multiple comparisons was also stressed in relation to sex ratio by McDowall (1985). The disadvantage of reducing the chance of false positives is the resulting increase of the chance of rejecting a real association. In this perspective it should be realised that the existence of supporting evidence for the apparent association and the consistency over time are important. McDowall (1985) stated in relation the studies between occupations and reproductive outcomes that a set of consistent but non-significant risks for a group of related occupations may be a better guide to a genuine problem than a single isolated statistically significant result.

The fruit growers study indicated that, besides some pesticides, there were also other factors which may determine extent of exposure such as spraying frequency and frequency of use of specific equipment, which were related to a shift towards more daughters within a family.

The evidence of a relation between occupational pesticide exposure and offspring sex ratio is except for the study on DBCP, non-existent. Also, no supporting evidence such as information from animal studies on specific chemicals is available. This study, therefore, can only suggest an interesting line of research for future studies.

Table A-1 Most commonly used pesticides in fruit growing in 1989 (n= 43 farms), according to percentage of farms (> 50%)

Pesticide	Farms(%)
1. captan (f)	98
2. simazine (h)	88
3. paraquat (h)	84
4. bitertanol (f)	67
5. glyfosaat (h)	60
6. MCPA (h)	60
7. carbaryl (i)	58
8. hexathiazox (a)	58
9. metiram (f)	56
10. broompropylaat (a)	53
11. thiram (f)	53
12. tolylfluanid (f)	53
13. azinphos-methyl (i)	53

f= fungicide; h= herbicide; i=insecticide; a=acaricide.

Table A-2 Most commonly used pesticides in fruit growing in 1989 (n = 43 farms), according to frequency of spraying (more than one spraying/y)

Pesticide	Sprayings/y
1. captan (f)	10.45
2. metiram (h)	2.70
3. tolylfluanide (h)	2.65
4. bitertanol (f)	2.47
5. thiram (f)	1.60
6. paraquat (h)	1.33
7. azinphos-methyl (i)	1.12

f= fungicide; h= herbicide; i=insecticide; a=acaricide.

Table A-3 Odds Ratio of 16 cases (more daughters than sons within a family) compared to 27 controls (43 families) for used pesticides (yes/no) in 1989

	Percentage of firms			
	cases	controls	OR	95%-CI
azinphos-methyl (i)	75	41	4.4	1.11-17.1
bitertanol (f)	69	67	1.1	0.29-4.14
broompropylaat (a)	56	52	1.2	0.34-4.14
captan (f)	100	96	>0.6	0.62-<24.6
carbaryl (i)	50	63	0.6	0.17-2.06
glyfosaat (h)	63	59	1.2	0.32-4.08
hexathiazox (a)	63	56	1.3	0.38-4.73
MCPA (h)	63	59	1.2	0.32-4.08
metiram (f)	75	44	3.8	0.96-14.7
paraquat (h)	100	74	>5.6	5.60-<13.4
simazine (h)	94	85	2.6	0.27-25.7
thiram (f)	63	48	1.8	0.51-6.34
tolylfluanide (f)	69	44	2.8	0.75-10.1

Table A-4 Odds Ratio of 16 cases (more daughters than sons within a family) compared to 27 controls (43 families) for number of spraying days per year (days/y) in 1989

	Days/year			
	cases	controls	OR	95%-CI
azinphos-methyl (i)	1.7	0.8	1.5	0.93-2.41
captan (f)	9.8	10.9	1.0	0.88-1.07
metiram (f)	4.4	1.7	1.3	1.05-1.61
paraquat (h)	1.5	1.2	1.4	0.70-2.80
thiram (f)	2.2	1.3	1.2	0.90-1.64
tolylfluanide (f)	3.4	2.2	1.1	0.92-1.31

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CHAPTER 8

General discussion and conclusions

Occupational exposure to pesticides in fruit growing is characterized by potential exposure to a cocktail of pesticides and remaining residues. Exposure may take place via the dermal, respiratory, and oral pathway. An important feature of exposure to pesticides is the great day-to-day variability. Exposure also may vary over the course of one year following seasonal variations in use or over a greater time span as a result of changes in pesticide usage. For many pesticides the dermal route of exposure may be predominant. Also the nature of exposure may vary for different tasks. Mixing and loading of tank mixtures concerns peak exposures of a short duration of time, partly to undiluted agents. Exposure during re-entry of the orchards is of longer duration and likely to be of a more constant nature during a working day. These features of exposure are important when designing hazard control strategies, for compliance with standards and in epidemiological studies. The advantage of this study in fruit growing compared to most studies on pesticide exposure is the relatively large number of exposure data and repeated measurements on the same subjects.

This discussion will focus first on the study population being representative for fruit growers in The Netherlands and secondly on captan being a good marker for exposure to pesticides in general. Next, the exposure measurements will be discussed followed by a discussion on the shown effects of pesticide exposure on reproduction. As the results of the exposure studies may form a basis for control of exposure, some general points for designing a control strategy are addressed briefly. Finally, general conclusions and recommendations are given.

STUDY POPULATION

A representative sample of an exposed population is seen as the 'gold standard' of exposure assessment strategies. An advantage of such a sample is that unbiased estimates of the population exposure may be obtained (Teschke *et al.*, 1994).

The main difficulties in achieving a representative sample are feasibility and costs. In order to achieve a representative sample of the fruit growers population a postal questionnaire was sent out in 1989 to all 2,730 members of the Dutch National Fruit Growers' Organization (NFO). The NFO covered over 90% of all fruit growing farms in The Netherlands (LEI 1992).

A non-response interview by telephone among randomly selected fruit growers revealed that the main reasons for not participating were that fruit growers thought the questionnaire was not applicable to their situation. Reasons mentioned were a small crop area, fruit growing was a sideline activity or because termination of farm business had already been planned. Further reasons were the high number of questionnaires that had to be returned in general, or expressing no interest in the subject. Farmers with a smaller crop area or planned farm termination, may consist partly of older farmers using older spraying techniques, which possibly may include farmers with a higher exposure during spraying.

For the exposure studies, fruit growers from the population who were willing to participate in further studies were selected from the three main fruit growing regions, South-West ('Zeeland'), Middle ('Betuwe') and the Polder area ('Flevopolders'). The farmers who were not willing to participate in further studies had a smaller crop area, used to a lesser extent integrated pest control, and fewer had followed a course on handling of pesticides (Table 1). These differences indicate that some of the farms with older farm practices and higher exposure during spraying were excluded.

Table 1 Differences between the response and non-response population and farms actual participating in field studies and the remainder of the responders

Responders *vs* Non-responders	Responders		Non-responders*	*P*-value
number of farms	915		141	
crop area (ha)	9		7	< 0.01[A]
integrated pest control	36%		23%	< 0.01[B]
course on handling pesticides	49%		40%	0.01[B]
Participation in field studies	**Yes**	**No**		
number of farms	106	809		
crop area (ha)	10	7.7		< 0.01[A]
course on handling pesticides	54%	43%		0.03[B]
number of captan sprayings in 1989	10	9		< 0.01[A]

* Random sample; [A] Median test, Chi square; [B] two tailed Fisher Exact test.

It is not likely that this influenced the study on time to pregnancy since most of the older farmers probably would have had children older then 12 years of age, which would have been excluded for that reason.

Actual participation in the exposure studies depended on a combination of use of *captan* and weather conditions on the day when a survey was carried out. The farms which actually participated differed on some points when compared to the rest of the 915 farms for which questionnaire data were available (Table 1). Missing the smaller farms to some extent, and a number of the farmers who had not followed a course on pesticide handling, may have resulted in underestimation of exposure.

It is not clear if exposure during re-entry is likely to differ for the non-responders compared to the measured farms. Dislodgeable foliar residue (DFR), the main determinant of exposure during re-entry, is related to the amount sprayed and the time elapsed since last spraying. The amount applied[1] is assumed to reflect the situation of fruit growing in The Netherlands. Representativeness of re-entry exposure is more likely to depend on how well the re-entry times observed reflect the general situation. In 1991 half of the workers entered lots within 8 days after spraying, although not for all individual

[1] approximately 900 g/ha on average; range 53 - 2075 g/ha

workers the time of re-entry since the last performed spraying was exactly known. During part of the year the spraying frequency is once within every two weeks, therefore, exposure during re-entry is likely to occur. We have no indications that the farms we visited deviated in this respect with what is to be expected in general. Furthermore, we are unable to state the differences between fruit farmers in the study and the 10% non-NFO members.

On balance, for pesticide application the exposure data on captan seems to be representative for hard fruit and the use of an airblast sprayer. For re-entry it depends on the time elapsed before re-entry. It is not certain if these data represent fruit growing in general in this respect.

MARKER FOR EXPOSURE

Exposure to pesticides in agriculture is characterized by mixed exposure. Therefore, it is not surprising that most epidemiological studies in agriculture usually link 'exposure to pesticides' as such to adverse health effects or even 'working in agriculture' as an even more rough surrogate for pesticide exposure. So far, very few epidemiological studies could identify specific compounds. In those cases it mainly concerned exposure of workers engaged in the production and formulation of pesticides.

As differences in physicochemical properties of pesticides not only have an effect on persistence in the orchard but also on uptake into the human body, metabolism, and excretion, extrapolation from one pesticide to another is complex. An empirical formula for estimating skin penetration as described by Fiserova-Bergerova (1993) therefore can only be a first approximation for pesticides which may have a potential for dermal uptake.

Extrapolations from external exposure measurements on captan to other pesticides can be made only if these pesticides concern chemical compounds with the same physicochemical properties, applied under the same conditions, such as a comparable type of farming, equipment used, type of formulation (powder or liquid) and climatological conditions. Captan may be a good representative for powdery fungicides applied as wettable powder by an airblast sprayer in fruit growing. Differences in decay in the orchards between pesticides, however, may be large. As a result, this may lead to differences in exposure between pesticides during re-entry work. The choice of captan made a large scale study with repeated measurements on the same workers possible and also a biological monitoring method to evaluate uptake into the body was available.

EXPOSURE

The main tasks studied were application of pesticides and re-entry tasks such as thinning, bending/tying up, pruning and harvesting. The main determinant for respiratory exposure during re-entry was dislodgeable foliar residue (DFR) in the orchard. For application of pesticides, including mixing/loading and spraying, respiratory exposure was explained predominantly by factors related to mixing and loading of undiluted captan dust. Mixing and loading (dust) also differ in nature of exposure compared with spraying (spray droplets).

Dermal exposure depends on performed tasks and on the considered skin locations. Differences in respiratory and dermal exposure levels between application and re-entry were, although statistically significant, not very large. Only for forehead, the difference was larger than one order of magnitude. Time spent per day on application of pesticides, however, is normally much shorter than time spent on re-entry tasks. The individual time spent on different tasks is often decisive for the total individual exposure. Highest dermal exposure per unit of time was observed during preparation of tank mixtures. As duration spent on this task is short, its contribution to the total dose depends on the number of prepared tank mixtures, how long the contamination is available for uptake, and whether the hands will contaminate other skin areas. Use of a cabin was the main determinant of dermal exposure during application. For re-entry tasks dislodgeable foliar residue (DFR) was most important.

The type of exposure, however, differs between these tasks. For the fruit grower who performs a spraying, part of the exposure will be exposure to peak levels during mixing and loading. For re-entry activities exposure duration will be longer and more constant. On the whole, seasonal variation in performed activities leads to intermittent exposures.

Variability

For exposure to pesticides in agriculture, data on its variability is scarce. Both environmental and production factors are recognized to influence the day-to-day component of variance for occupational exposure in general and to a much lesser extent the between-worker component of variance (Kromhout, 1994).

Variability in exposure from repeated measurements generally was high within and between workers. For application, the number of repeated measurements on the same subjects was very small. For re-entry work in the orchards subjects were not uniformly exposed from day to day. Therefore, to estimate individual exposure accurately, repeated measurements on the same subject are necessary.

As dislodgeable foliar residue (DFR) was the most prominent determinant of exposure and DFR depends on amount sprayed and time elapsed since last spraying, repeated measurements of DFR may be used as an estimate of individual exposure. The suggestion (Popendorf and Leffingwell, 1982; Nigg *et al.*, 1984; Zweig, 1985) that a fixed relation exists between DFR and dermal exposure (a transfer factor) is attractive, but may not be true in general. It was shown for instance that fruit picking rate influenced exposure (Fenske, 1989) and the transfer factor in our study was lower than found for fruit pickers by Zweig *et al.* (1985).

Determinants

Fixed determinants of exposure, which are constant over time, with a strong influence on exposure may be used for grouping workers according to long term exposure. Such determinants, however, may only be valid for one specific exposure measure. For instance, cabin use during spraying had a strong influence on dermal exposure of forehead, but exposure of hands and respiratory exposure was similar for cabin and non-cabin users. During re-entry, however, the opposite was seen, since exposure of forehead was not explained by DFR, whilst exposure of the hands was.

As determinants of exposure vary for the different exposure routes and body locations (dermal exposure), the measure of interest for a specific study design will decide which determinants are most relevant in a study.

As the population of fruit growers can be grouped according to determinants of potential external exposure, knowledge of the determinants affecting the exposure can be used for control strategies to reduce exposure and in epidemiological studies.

Occupational exposure limits

Standards for occupational exposure levels for respiratory exposure are not applicable in situations where other exposure routes are predominant. As the occupational exposure limit (OEL) is set on the basis of data for respiratory exposure, one can argue that in a situation where the dermal route predominates and the respiratory route is of minor importance, the OEL is not a valid starting point to derive a "skin notation". The development of standards explicitly for dermal exposure seems more appropriate.

Unmeasured exposure

As exposure was only measured during spraying of captan on hard fruit with an airblast sprayer pulled by a tractor, we lack exposure data in relation to other spraying equipment.

The main work in fruit growing concerned hard fruit (*i.e.* apples and pears). Exposure to pesticides in small fruit (*i.e.* berries) on 13% of the farms is likely to be different from hard fruit, because of different working conditions, differences in pesticides used, and amounts applied (Plantenziektekundige Dienst, 1993), and was not studied. In the re-entry studies, we focused also explicitly on hard fruit.

For 43% of the farms, fruit farming is not the only activity. How personal exposure in fruit growing relates to pesticide exposure in other farm activities cannot be answered here.

As stated by Loomis and Savitz (1994) failure to include all exposure sources can bias results of epidemiological studies. Unmeasured exposure from non-fruit growing exposure and residential exposure is not likely to be a constant proportion of the measured exposure. Therefore, it is not possible to predict if this will result in bias of observed dose-response in upward or downward direction.

In conclusion, especially in situations where work environment and residential environment are closely connected, as in fruit growing, more attention should be paid to the contribution of residential and non-work related exposures.

EFFECTS ON REPRODUCTION

The recognition that exposure to pesticides may be significant for workers in agriculture, and that a relationship may exist with effects on human reproduction has only recently been considered (Baker *et al.*, 1990; Restrepo *et al.*, 1990; WHO 1986, 1988). For many pesticides, no information is available on possible reproductive effects in human. The lack of data about complex exposures in agriculture and possible inconsistencies between animal studies and human epidemiological data gave rise to an explorative epidemiological study. Our results indicated an adverse effect of exposure to pesticides on fecundability. It is, however, impossible to draw conclusions about specific pesticides responsible for the effect, or about the underlying mechanism.

Exposure determination

Initially, data from questionnaires roughly reflecting working conditions, which indicated exposure of the male, were studied. The determinants application of pesticides by the farm owner and spraying velocity, seemed to be independent indicators of exposure. Application of pesticides solely by the farm owner most probably leads to a longer duration of contact with pesticides each season, compared with farmers who share this task with other persons. Spraying velocity is assumed to be a good indicator of intensity of exposure, because a low spraying velocity probably leads to a more intensive contact with pesticides.

Exposure measurements from field studies carried out in the same year supported the validity of the exposure indices used. Some of the underlying variables of spraying velocity have shown a clear relation with exposure. The effect of a cabin on dermal exposure was confirmed by exposure measurements during application of captan. The exposure variables application of pesticides by the farm owner and spraying velocity are directly related to the exposure of the applicator. Usually mixing, loading, and applying of pesticides is a task exclusively of the men.

It is impossible to conclude if other unmeasured sources of exposure might have had an influence on the findings. For the fruit grower only detailed information on the application of pesticides was available on an individual level at that time and not on re-entry tasks.

Alternative explanations

It is not possible to pinpoint whether it is only the male exposure that affects reproduction. A possible alternative explanation is a higher degree of participation of the wife in farm work resulting in exposure when working in the orchard. For both the high and low exposure categories for male, the type of work performed by the wives was the same. Unfortunately, no information was available on individual time spent on the farm by the wives, so a definite answer cannot be given. The male tends to spend more time on application of pesticides and other orchard tasks. Thus, his exposure is likely to be higher and the relation of reproductive effects with the male more plausible. This is supported by the finding that application of pesticide by the owner (*i.e.* male fruit grower) and spraying velocity seemed to be independent indicators of exposure, by which the farmers could be grouped.

In general, it has been recognized that peak exposures may also cause adverse effects, that may be distinct from those of cumulative exposure (Stewart, 1991). Therefore, depending on the tasks performed, the same cumulative exposure may yield differences in risk for individual workers. As application of pesticides include mixing and loading, which is a task of the male, exposure to peak exposures were likely to occur for the male and not for the female.

It is unlikely that the increased time to pregnancy of couples trying to conceive during the spraying season is due to indirect effects such as high work load, stress, or a decreased frequency of sexual intercourse, because time to pregnancy was not increased during the spraying season for the farmers with a low exposure.

Later exposure studies during re-entry showed that exposure per unit of time during re-entry was in the same order of magnitude compared with application and that individual time spent on these tasks is crucial for estimating individual exposure.

As the exposure indices used were indirect measures of exposure, which could not be validated on an individual level, future studies should focus on individual time spent on both application and re-entry tasks of male and female. Biomonitoring may be used to validate the relevance of exposure measures or determinants to model exposure.

Offspring sex ratio

There are some slight indications that exposure to pesticides in fruit growing may affect offspring sex ratio as well. For future studies it seems useful to explore if both sex ratio and time to pregnancy are interlinked by calculating time-to-boy and time-to-girl, separately. The presence of families with a predominance of one of the sexes would also be interesting to study as little is known about the underlying biological mechanisms or the statistical properties of these indices.

In conclusion, both time to pregnancy and sex ratio may be used as simple and sensitive measures in epidemiological studies to serve as early warning signs for potential adverse effects of pesticide exposure. As both male and female may be exposed, more attention in future studies should be given to characterizing individual exposure to pesticides.

CONTROL STRATEGIES

In occupational hygiene, elimination of the source is the control measure of first choice. For occupational exposure to pesticides this approach conflicts with the purpose of pesticide use, as pesticides are intentionally spread on crops. The fruit grower has some influence on the type of equipment used, the chemicals, the formulations and concentrations. Control strategies, however, generally are concentrated on avoiding exposure and on personal protection.

Awareness of exposure may play an important role. It was shown that fruit growers are aware of exposure in some respect during the application. Level of awareness, however, may differ for the fruit grower and workers in the orchards.

Findings from this study may form a basis to advice on hazard control to reduce exposure in fruit growing. Starting points for control strategies should be based on the main determinants of exposure. These are the cabin during spraying, dislodgeable foliar residue (DFR) during re-entry, and individual time spent on different tasks.

During spraying, the strength of the observed effects of cabin use, however, may have been influenced partly by awareness of fruit growers. This may also apply for glove use. Fruit growers may feel 'safe' when using gloves. Therefore, it will be essential to perform intervention studies to evaluate the 'real' effectiveness after introduction of measures to reduce exposure.

For re-entry, knowledge on the pesticides used and time elapsed since last spraying are important in reducing exposure when working in the orchards. The sooner someone enters the orchard after a spraying, the higher the potential exposure may be. For a proper evaluation, data are needed on decay of the different pesticides used under the actual fruit growing conditions. As the half-life of captan on crops may vary from 10-17 days, substantial exposure due to early entry to the orchards is likely to occur. To be able to evaluate whether re-entry intervals are desirable for some pesticides, studies on exposure level during re-entry to the different pesticides are advisable since generally no data are available.

Special attention should be paid to mixing and loading. Use of powdery products may cause peak levels of respiratory and dermal exposure to the concentrated chemicals. Skin contamination from preparing tank mixtures potentially will be available for uptake into the body for a longer duration of time than the time spent on the preparation of tank mixtures. Exposure of the hands also can form a secondary source of contamination for other parts of the skin and may even cause oral uptake indirectly.

Experts recognize the most important determinants of external exposure and therefore may play a role in evaluating exposure.

In conclusion, a control strategy should cover:

1) Increasing *awareness* of potential exposure to all workers;
2) Using a *tractor cabin* for the application of pesticides with an airblast sprayer;
3) Systematic sampling of *data on decay* of the most commonly used, or otherwise relevant pesticides under actual circumstances in fruit growing;
4) Considering the need for *re-entry intervals* for specific pesticides under specific conditions;
5) Evaluating the *effectiveness and feasibility* of measures taken to reduce occupational pesticide exposure;
6) Giving regular *feedback* of the findings to the fruit growers.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

1) Repeated measurements are necessary to estimate individual exposure accurately, as day-to-day variability in exposure was high.
2) A reduction of exposure to pesticides as far as achievable is needed, as the current exposure levels in fruit growing appear to have an effect on reproduction.
3) Control measures should be proposed to fruit growers, based on the main determinants of exposure. Furthermore, the effectiveness of the introduced measures should be evaluated.
4) Data on decay of the different pesticides used should be measured to assess under what circumstances re-entry intervals may be necessary and if re-entry intervals are feasible.
5) Experts seem to recognize the most important determinants of external exposure, and therefore may play a role in evaluating exposure.
6) The skin as exposure route may play a predominant role for many pesticides. As this role is not expressed in the way standards for occupational exposures are set, a systematic strategy to develop standards for dermal exposure is needed.
7) Dermal exposure data can be linked better to biological monitoring based on empirical findings as gathered in a pilot study on exposure of specific body areas than on estimations of total skin dose.
8) The findings of the study on time to pregnancy indicate that an adverse effect of pesticide exposure is likely. The indications justify more attention for possible effects of pesticide exposure on reproduction. In future studies more attention should be given to exposure during re-entry and individual exposure of both male and female.
9) The use of sex-ratio as a measure of effect may be relevant, but the evidence in the literature is weak and will first need to be explored further. It might be of interest to consider if sex ratio and time to pregnancy are interlinked and are sensitive measures to combine in studies on possible effects of occupational exposures on reproduction.

10) Further research on the causality and underlying mechanisms should not be restricted to epidemiological studies. More information on the toxicological repro-toxic properties of the different chemicals is needed to evaluate if the observed effects are attributable to a specific chemical or combinations thereof.

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Glossary

ACGIH	American Conference of Governmental Industrial Hygienists
ADI	Acceptable Daily Intake
CBS	Centraal Bureau voor de Statistiek
DBCP	Dibromochloropropane
DECOS	Dutch Expert Committee on Occupational Standards
DFR	Dislodgeable Foliar Residue
DGA	Directorate General of Labour, Ministry of Social Affairs and Employment
DOEL	Dermal Occupational Exposure Limits
Dose	Amount of uptake into the body
EC	European Community
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EPA	Environmental Protection Agency
Exposure	Contact during mixing/loading and/or spraying or during re-entry or other work activities on the farm.
External exposure	Potential exposure, *i.e.* the amount of pesticides available for exposure, of skin and/or respiratory tract, not considering protection by factors as, for example, personal protection equipment.
FAO	Food and Agriculture Organization
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
HPLC	High Performance Liquid Chromatography
IKC	Information and Knowledge Centre
IMAG	DLO-instituut voor Milieu en Agritechniek; Institute of Agricultural and Environmental Engineering
ISZW	Inspectie Sociale Zaken en Werkgelegenheid
Landbouwschap	Board of Agriculture
LD50	Lethal Dose for 50% of test animals
LEI	Landbouw-Economisch Instituut (LEI-DLO)
MAC	Maximum Accepted Concentration
MRL	Maximum Residue Limit
NRC	National Research Council
NFO	Nederlandse Fruittelers Organisatie; Dutch National Fruit Growers' Organization
OEL	Occupational Exposure Limit
PGF	Produktschap voor Groenten en Fruit; Product Board for Vegetables and Fruit
Re-entry	Tasks performed in the orchards other than applications

STIGAS	Stichting Gezamenlijke Arbo Services; Foundation of Joint Services for Occupational Safety and Health
SR	Sex Ratio
THPI	Tetrahydrophtalimide
TTP	Time to Pregnancy
TLV	Treshold Limit Value
WHO	World Health Organization

Summary

Exposure to pesticides of fruit growers and effects on reproduction: an epidemiological approach

In this thesis exposure to pesticides of fruit growers in The Netherlands was studied as well as its relation to reproductive health effects. Several exposure studies were carried out to evaluate dermal and respiratory exposure. Also uptake into the body was evaluated and a method was described to estimate exposure with panels of experts. Possible effects of exposure on human reproduction were studied by using time to pregnancy and offspring sex ratio as outcome measures.

Chapter 1 presents the background of this study. There is some concern that chronic healths effects may occur as a result of relatively high occupational exposure to pesticides. Fruit growing was chosen, since data on exposure for this sector were not available. Furthermore, because fruit growers tend to use a large number of different pesticides frequently and in considerable amounts. Based on findings in patients visiting the fertility clinic, physicians of the Academic University Hospital of Utrecht raised the question if pesticide exposure may lead to effects on reproduction. Therefore, this study also focused on reproductive effects.

Chapter 2 describes the exposure to pesticides of the study population. Large scale exposure surveys in fruit growing in The Netherlands were carried out during application of pesticides among 94 farmers and during re-entry tasks among 138 persons working in orchards. For 61 workers repeated measurements on the same subject were obtained. Additionally, pesticide contamination levels inside the homes of 10 fruit growers were measured.

In this study exposure to pesticides has been described in terms of average exposure levels and variability in measured concentrations. The relative contribution of the respiratory route and different skin sites to total exposure has been assessed. Captan was used as a marker for exposure. Also, total exposure for the fruit grower and his family members during the growing and harvesting season was estimated.

Inhalable dust exposure was measured with a personal air sampler and dermal exposure with skin pads and hand rinsing. Dislodgeable foliar residue (DFR) was measured by taking leaf punches from foliage in the orchards.

Between respiratory exposure and dermal exposure of hands, wrists and forehead, differences were observed between several tasks. Grouping workers according to performed tasks, therefore will depend on the exposure measure(s) (*e.g.* hands, forehead, inhalable dust), which are considered to be relevant for a specific study purpose. As exposure per unit of time was in the same order of magnitude for different tasks, individual time spent on these tasks is crucial for estimating total exposure.

In general, within-worker variability of all exposure measures was larger relative to between-worker variability. Therefore, repeated measurements are necessary to estimate individual exposure accurately. Variability in dermal exposure on the same body location was small relative to variability between different body locations.

Differences in total exposure, including exposure inside home, between the fruit grower and the son were small. Exposure of the wife was around 2-3 times lower than for the fruit grower and the son. To estimate internal exposure dose for epidemiological purposes and risk assessment, the key element is ascertaining accurate information on the contribution of the specific exposure routes to the effective target dose.

Chapter 3 describes the determinants of exposure. A series of studies has been carried out on occupational exposure to pesticides among fruit growers in The Netherlands during spraying and re-entry of the crops between 1990 and 1992 to quantify exposure and identify determinants of exposure. Determinants of exposure are discussed as a starting point for hazard identification and control *i.e.* measures to be taken to reduce occupational exposure to pesticides.

Cabin use on the tractor was the most prominent determinant of dermal exposure during spraying. Dermal exposure studied in subgroups according to cabin use was related to determinants of exposure, which varied for different body sites. For respiratory exposure, factors related to preparation of pesticides were most prominent.

A long duration of exposure may reflect a different exposure situation compared with a short duration of exposure. As different determinants of exposure prevailed for both subgroups, it should be considered to construct exposure models for both groups separately.

Dislodgeable foliar residue (DFR) was the most prominent determinant of exposure for both respiratory and dermal exposure during re-entry. However, no significant relation between DFR and dermal exposure of forehead and sternal area was found. This may be explained by indirect exposure of these skin areas as compared with direct contact of other body parts such as hands and arms with foliage. Therefore, it was concluded that use of a transfer factor based on dislodgeable foliar residue to estimate total dermal exposure is only a crude estimate.

The half-life of captan on crops varied from 10 - 17 days, and so substantial exposure when entering the orchard is very likely to occur, particularly when spraying frequency is high.

The main starting points for reduction of exposure are use of a cabin, dislodgeable foliar residue and individual time spent on different tasks. Especially determinants which are constant over time (cabin use) may have a great influence on grouping workers according to long term exposure in epidemiological studies.

As determinants of exposure vary for the different exposure routes and body locations (for dermal exposure), the measure of interest for a specific study design will decide which determinants are most relevant.

Chapter 4 describes the subjective assessment of pesticide exposure by experts. Exposure to pesticides in fruit growing was estimated by experts with different professional expertise, respectively pesticide experts, occupational hygienists and fruit growing experts to see whether valid subjective assessments can be made by experts. The study objectives were: 1) validation of exposure assessment by experts using different sources of information;

2) assessment of inter-rater agreement; 3) measurement of agreement between experts' assessment and actual quantitative exposure data.

An expert panel of three groups with different expertise, made four ratings. For three of these ratings assessments were made in three phases in each of which exposure information was provided.

Intra-class correlation was high for each subgroup of experts when tasks in fruit growing were relatively ranked by increasing exposure level. In general, inter-rater agreement on factors influencing the internal dose decreased when more information on exposure was provided.

All experts correctly considered dermal exposure as the prominent contributor to internal dose. Results were comparable for three pesticides under study. Ranking of actual exposure situations of 15 specific sprayings with a fungicide, clearly showed differences between raters according to their expertise. The pesticide experts and occupational hygienists were able to rank daily exposure levels during pesticide spraying in a meaningful way.

The experts seem to recognize the most important determinants of external exposure and therefore may play a role in evaluating exposure. Preferably, an expert panel should not be too small, and consensus or average estimates should be used, because differences within expert groups can be considerable.

Chapter 5 describes the urinary excretion of a metabolite of captan after exposure. The relation between dermal and respiratory exposure and uptake into the body of captan, measured as 24 hr cumulative tetrahydrophtalimide (THPI) dose, was studied among 14 male fruit growers applying pesticides in orchards in The Netherlands. No contribution of respiratory exposure was observed on THPI in the urine. Dermal exposure, measured with skin pads, showed a clear relation with THPI in urine when exposure was estimated from exposure on skin pads of ankles and neck. No relation was found for total dermal exposure, calculated from measured exposure on skin pads of representative skin areas according to models described in the literature. Determinants of exposure such as use of a cabin on the tractor, use of gloves during mixing and loading, and use of rubber boots also explained THPI in urine very well. This finding corroborated the findings on measured dermal exposure. Results indicate that more attention should be paid to skin areas which are suspected to be most permeable for a chemical under study.

It was concluded that dermal exposure data can be linked better to biological monitoring based on empirical findings as gathered in a pilot study on exposure of specific body areas than on estimations of total skin dose. Based on the findings, that the dermal route predominates and the respiratory route is of minor importance, the occupational exposure limit for respiratory exposure is not a valid starting point to derive a "skin notation". The development of standards explicitly for dermal exposure seems more appropriate.

Chapter 6 describes the relationship between time to pregnancy and pesticide exposure. Although pesticides are regularly used in agriculture, relatively little is known about possible adverse health effects, especially reproductive effects, due to occupational exposure. This explorative study investigates the relation between exposure of the fruit grower to pesticides and fecundability (probability of pregnancy) in a population of fruit growers.

The analysis is based on self reported data and includes 91 pregnancies during 1978-1990 of 43 couples. Cox' proportional hazards model was used to analyse time to pregnancy after correction for gravidity and consultation with a physician for fertility problems.

Application of pesticides solely by the owner was associated with a long time to pregnancy, resulting in a fecundability ratio of 0.46 (95% confidence interval (95% CI) 0.28-0.77). Similarly a low spraying velocity ($\leq$ 1.5 hectares/h) resulted in a fecundability ratio of 0.47 (95% CI 0.29-0.76) and is associated with the use of older spraying techniques and tractors without a cabin. These factors were assumed to cause high exposure, which was confirmed by exposure measurements in the field. The effect of high exposure was mainly apparent if the couple had intended to become pregnant in the period from March-November (fecundability ratio 0.42, 95% CI 0.20-0.92). This is the period in which pesticides are applied. Out of the spraying season the effect of a high exposure was absent (fecundability ratio 0.82, 95% CI 0.33-2.02). In the high-exposure group 28% of the pregnancies had been preceded by consulting a physician because of fertility problems, compared to 8% in the low-exposure group. These findings indicate that an adverse effect of exposure to pesticides on fecundability is likely.

Chapter 7 describes the use of offspring sex ratio as an indicator of reproductive hazards due to pesticide exposure. The hypothesis that human offspring sex ratio (male/female ratio) might be affected by environmental or occupational factors is not new, but the evidence is weak.

The underlying mechanisms have not yet been elucidated. Effects on chromosome level, resulting in death in utero for the male foetus has been suggested. Other evidence points to a possible influence of hormone levels of the parents at the time of conception. Several larger studies on deviations in sex ratio have shown inconsistent results. Some other studies were hampered by small study populations, poor estimation of individual exposure or poor control for the many potential confounders.

An advantage of offspring sex ratio as a marker of possible health effects is that it is in principle unbiased and easy to monitor through vital records. If sensitive, this measure may serve as a warning sign for potential adverse effects of environmental or occupational exposures.

In reply to a letter to the editor on our study on time to pregnancy, offspring sex ratio was studied in the same population. From our results among fruit growers we concluded that it seems useful to study further if sex ratio is linked to pesticide exposure and if both sex ratio and time to pregnancy are interlinked.

In **Chapter 8** a general discussion is given. For the application of pesticides the exposure data seem to be representative for hard fruit and the use of an airblast sprayer. For re-entry it depends on the time elapsed before re-entry. It is not certain if these data represent fruit growing in general in this respect.

Captan was considered a good representative for powdery fungicides applied as wettable powder by an airblast sprayer in fruit growing. Differences in decay in the orchards between pesticides, however, may be large. As a result this may lead to differences in exposure during re-entry and therefore additional information on decay for other pesticides in the orchards is needed. As human data lack on uptake into the body for most pesticides, a comparison on uptake of captan with other compounds is not possible.

From the findings of the different exposure studies it was concluded that, since day-to-day variability in exposure was high, repeated measurements are necessary to estimate individual exposure accurately.

As determinants of exposure vary for the different exposure routes and body locations (dermal exposure), the exposure measure of interest for a specific study design will decide which determinants are most relevant.

As the population of fruit growers can be grouped according to determinants of potential external exposure, knowledge of the determinants affecting the exposure can be used for control strategies to reduce exposure and in epidemiological studies.

Dermal absorption occurred from ankles and the neck without an obvious contribution of the respiratory route. Therefore, in a situation where the dermal route predominates and respiratory exposure is of minor importance, an occupational exposure limits, which is based on respiratory exposure, is not a valid starting point to derive a "skin notation" in itself.

As unmeasured exposure, *i.e.* failure to include all exposure sources, can bias results of epidemiological studies more attention is necessary for the contribution of residential and non-work related exposure.

As not many epidemiological studies have been carried out on the effects of pesticides on reproduction, no firm conclusions can be drawn about the pesticides or other related factors responsible for the observed effect on time to pregnancy. First, these findings will need further confirmation. The validity of the observed effects on reproduction and the underlying mechanisms have to be elucidated. The use of sex ratio as a measure of effect may be relevant, but the evidence in literature is weak and will have to be explored further. Both time to pregnancy and sex ratio may be used as simple and sensitive measures in epidemiological studies to serve as early warning signs for potential adverse effects of pesticide exposure. As both male and female may be exposed, more attention in the future should be given to characterizing individual exposure to pesticides.

The indication that current exposure in fruit growing may have an effect on reproduction justifies more attention on possible effects of pesticide exposure on reproduction and on reduction of exposure.

Samenvatting

Blootstelling van fruittelers aan bestrijdingsmiddelen en effecten op de reproduktie: een epidemiologische benadering

In dit proefschrift wordt een studie beschreven naar de beroepsmatige blootstelling van fruittelers in Nederland aan bestrijdingsmiddelen in relatie tot reproduktie. Verschillende blootstellingsstudies zijn uitgevoerd om de blootstelling van de huid en de luchtwegen te evalueren. Ook de opname van bestrijdingsmiddelen in het lichaam werd bestudeerd. Daarnaast werden methoden geëvalueerd om de blootstelling aan bestrijdingsmiddelen te schatten met panels bestaande uit deskundigen. Mogelijke effecten van blootstelling op de menselijke reproduktie werden bestudeerd door tijd tot zwangerschap en de geboorte sekse verhouding (verhouding jongens/meisjes) als effectmaat te gebruiken.

Hoofdstuk 1 belicht de achtergronden voor het tot stand komen van dit onderzoek. Er bestaat enige bezorgdheid dat chronische gezondheidseffecten kunnen voorkomen als gevolg van relatief hoge beroepsmatige blootstelling aan bestrijdingsmiddelen. De fruitteelt werd gekozen, omdat er voor deze sector nog geen duidelijk beeld bestond over de blootstelling. Bovendien wordt in deze sector frequent en in aanzienlijke hoeveelheden een groot aantal bestrijdingsmiddelen gebruikt. Een vraag van het academisch ziekenhuis in Utrecht, gebaseerd op ervaringen met patiënten, of het gebruik van bestrijdingsmiddelen kan leiden tot onvruchtbaarheid, richtte de aandacht van het onderzoek op de mogelijke effecten op de reproduktie.

Hoofdstuk 2 beschrijft de blootstelling van de studiepopulatie aan bestrijdingsmiddelen. Grootschalige blootstellingsstudies in de fruitteelt in Nederland zijn uitgevoerd tijdens het verspuiten van bestrijdingsmiddelen door 94 fruittelers en tijdens re-entry[1] taken door 138 personen. Voor 61 personen werden meerdere waarnemingen per persoon verzameld. Tevens werd in 10 woningen van fruittelers het besmettingsniveau aan bestrijdingsmiddelen gemeten.

In deze studie werd het gemiddelde blootstellingsniveau en de variabiliteit in blootstelling beschreven. De relatieve bijdrage van de luchtwegen en de verschillende huiddelen aan de totale blootstelling werd geschat. Captan werd gebruikt als modelstof voor de blootstelling. Ook de totale blootstelling van de fruitteler en zijn gezinsleden tijdens het groei- en oogstseizoen werd geschat.

De persoonlijke luchtwegblootstelling aan inhaleerbare deeltjes werd gemeten met een draagbaar pompje. De huidblootstelling werd bepaald met huidpads en met behulp van een handenspoelmethode. Bladresidu werd vastgesteld door het nemen van bladmonsters.

Er werden verschillen tussen werkzaamheden gevonden voor de luchtwegblootstelling en voor de huidblootstelling van handen, polsen en voorhoofd. Het groeperen van personen op basis van uitgevoerde werkzaamheden hangt daarom af van welke van deze blootstellingsmaten voor een specifiek onderzoeksdoel als relevant worden beschouwd. Aangezien de blootstelling uitgedrukt per tijdseenheid van dezelfde orde van grootte was voor verschillende werkzaamheden, is de individuele tijd die aan de werkzaamheden wordt besteed van doorslaggevend belang voor het schatten van de totale blootstelling.

In het algemeen was de variabiliteit voor de blootstellllingsvariabelen binnen personen relatief groter dan tussen personen. Daarom zijn herhaalde waarnemingen nodig om de individuele blootstelling nauwkeurig te schatten. De variabiliteit van de huidblootstelling binnen eenzelfde gedeelte van de huid was relatief klein ten opzichte van de variabiliteit tussen verschillende huiddelen.

Verschillen in de totale blootstelling tussen de fruitteler en de zoon, waarbij ook de blootstelling binnenshuis in aanmerking werd genomen, waren klein. De blootstelling van de vrouw lag ongeveer 2-3 keer lager dan voor de fruitteler en de zoon. Om de inwendige blootstellingsdosis te schatten in epidemiologische studies en risico-analyses, is nauwkeurige informatie over de bijdrage van de specifieke blootstellingsroutes essentieel.

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[1] Re-entry, of herbetreding zijn werkzaamheden in de boomgaard, anders dan het uitvoeren van een bespuiting

Hoofdstuk 3 beschrijft de determinanten van de blootstelling. In de periode 1990-1992 werd een aantal studies uitgevoerd naar de beroepsmatige blootstelling van fruittelers in Nederland aan bestrijdingsmiddelen tijdens het uitvoeren van bespuitingen en werkzaamheden in het gewas. Het doel was de blootstelling te kwantificeren en de factoren vast te stellen die de blootstelling bepalen. De factoren die de blootstelling bepalen worden besproken in het kader van risico identificatie en beheersstrategieën (maatregelen om de beroepsmatige blootstelling aan bestrijdingsmiddelen te reduceren).

Het gebruik van een cabine op de tractor was de duidelijkste factor die de huidblootstelling tijdens het uitvoeren van bespuitingen bepaalde. De huidblootstelling bestudeerd in subgroepen naar cabine gebruik was gerelateerd aan determinanten van blootstelling die varieerde voor de verschillende delen van de huid. Voor de luchtwegblootstelling waren factoren die te maken hadden met het aanmaken van een spuitmengsel het belangrijkst.

Een lange blootstellingsduur weerspiegelt mogelijk een andere blootstellingssituatie dan een korte blootstellingsduur. Aangezien de determinanten van de blootstelling die overheersten verschillend waren in de subgroepen met een verschillende blootstellingsduur, dient overwogen te worden om voor beide groepen afzonderlijk een model voor de blootstelling te beschrijven.

Bladresidu was de meest uitgesproken factor die zowel de luchtweg- als de huidblootstelling bepaalde tijdens re-entry. Er werd echter geen significante relatie gevonden tussen bladresidu en de huidblootstelling van voorhoofd en borst. Dit wordt mogelijk verklaard door de indirecte blootstelling van deze huiddelen in vergelijking met het directe contact met het gewas van andere huiddelen zoals handen en armen. Het gebruik van een overdrachtsfactor (*'transfer factor'*) om de totale huidblootstelling te benaderen is daarom slechts een ruwe benadering.

Aangezien de halfwaarde tijd van *captan* op het gewas varieerde van 10-17 dagen, is het waarschijnlijk dat een substantiële blootstelling optreedt wanneer het gewas wordt betreden, zeker als de spuitfrequentie hoog is.

De belangrijkste uitgangspunten om de blootstelling te reduceren zijn het gebruik van een cabine, de hoeveelheid residu op het gewas en de individuele tijd die wordt besteed aan de verschillende werkzaamheden. In het bijzonder factoren die constant zijn over de tijd (cabine op de tractor) kunnen een grote invloed hebben op het groeperen van werkers naar chronische blootstelling in epidemiologische studies.

Omdat de factoren die de blootstelling bepalen variëren voor de verschillende blootstellingsroutes en delen van het lichaam (voor de huidblootstelling), zal het van de specifieke studie opzet afhangen welke determinanten het meest relevant zijn.

Hoofdstuk 4 beschrijft het schatten van blootstelling aan bestrijdingsmiddelen door deskundigen. Het betrof deskundigen met verschillende expertise: bestrijdingsmiddelen-deskundigen, arbeidshygiënisten, en fruitteelt-deskundigen. Nagegaan werd of deskundigen in staat zijn betrouwbare subjectieve schattingen te maken. De doelstellingen waren: 1) validering van blootstellingsschatting door deskundigen; 2) schatting van overeenstemming tussen deskundigen; 3) vaststellen van de overeenstemming tussen de schatting van de deskundigen en kwantitatieve blootstellingsgegevens.

Een panel van deskundigen, bestaande uit de drie genoemde groepen, maakte vier verschillende schattingen. Drie schattingen werden gemaakt in drie verschillende fasen. In elke fase werd informatie over de blootstelling verstrekt.

De overeenstemming binnen elke subgroep van deskundigen was hoog als de taken in de fruitteelt werden gerangschikt naar toenemend blootstellingsniveau. Voor het schatten van factoren die de interne dosis beïnvloeden nam de overeenstemming tussen de deskundigen af naarmate er meer informatie over de blootstelling werd verstrekt.

Alle deskundigen beschouwden terecht de huidblootstelling als de factor van doorslaggevend belang voor de interne dosis. De resultaten waren vergelijkbaar voor drie bestudeerde bestrijdingsmiddelen. Het rangschikken van de feitelijke blootstelling van 15 specifieke bespuitingen met een fungicide liet duidelijk verschillen zien tussen deskundigen al naar gelang hun deskundigheid. De bestrijdingsmiddelen-deskundigen en de arbeidshygiënisten waren in staat de dagelijkse blootstellingsniveaus op een betekenisvolle wijze te rangschikken.

De deskundigen lijken de belangrijkste determinanten van de externe blootstelling te herkennen en kunnen daarom een rol spelen bij de evaluatie van de effectiviteit van beheersmaatregelen die bedoeld zijn om de externe blootstelling te reduceren. Bij voorkeur dient een deskundigen panel niet te klein te zijn en dienen 'consensus data' of groepsschattingen te worden gebruikt, omdat verschillen binnen de groep deskundigen aanzienlijk kunnen zijn.

Hoofdstuk 5 beschrijft de uitscheiding van een afbraakprodukt van *captan* na blootstelling via de urine. De relatie tussen huid- en luchtwegblootstelling en opname van *captan* in het lichaam, gemeten als de cumulatieve 24-uurs dosis tetrahydrophtalimide (THPI), werd bestudeerd bij 14 mannelijke fruittelers in Nederland na het uitvoeren van bespuitingen in de boomgaard. Er werd geen bijdrage van de luchtwegblootstelling gevonden op de hoeveelheid THPI in de urine. De huidblootstelling gemeten met huidpads, liet een duidelijke relatie zien met THPI in urine als de blootstelling werd geschat op basis van de blootstelling van de enkels en de nek. Er werd geen relatie gevonden met de totale huidblootstelling, berekend op basis van huidpads van representatieve huiddelen volgens modellen beschreven in de literatuur. Determinanten voor de blootstelling zoals het gebruik van een cabine op de tractor, het gebruik van handschoenen tijdens het aanmaken van een tanklading en het gebruik van rubberen laarzen verklaarde THPI in urine ook erg goed. Deze bevinding was in overeenstemming met de gemeten huidblootstelling. De resultaten geven aan dat er meer aandacht uit dient te gaan naar de huiddelen waarvan wordt verwacht dat ze het meest doorlatend zijn voor de bestudeerde chemische stof.

Geconcludeerd werd dat huidblootstelling beter gerelateerd kan worden aan biologische monitoring op grond van empirische bevindingen op basis van verkennende studies gericht op specifieke delen van de huid, dan op schattingen van de totale dosis. Uitgaande van de bevinding, dat de huid als blootstellingsroute overheerst en de luchtwegen van ondergeschikt belang zijn, is de grenswaarde voor beroepsmatige blootstelling voor de luchtwegen niet het juiste uitgangspunt om een huidindicatie vast te stellen. Het ontwikkelen van grenswaarden expliciet voor huidblootstelling lijkt een beter uitgangspunt.

Hoofdstuk 6 beschrijft de relatie tussen tijd tot zwangerschap en de blootstelling aan bestrijdingsmiddelen. Hoewel bestrijdingsmiddelen regelmatig worden gebruikt in de agrarische sector, is er relatief weinig bekend over mogelijke nadelige gezondheidseffecten, in het bijzonder als het gaat om effecten op de reproduktie als gevolg van beroepsmatige blootstelling. Deze verkennende studie onderzoekt de relatie tussen de blootstelling van de fruitteler aan bestrijdingsmiddelen en de fecundabiliteit (de kans op zwangerschap) in een populatie fruittelers.

De analyse is gebaseerd op zelf-gerapporteerde gegevens over 91 zwangerschappen in de periode 1978-1990 van 43 fruittelers paren. Het *Cox' proportional hazards model* werd gebruikt om de tijd tot zwangerschap te bestuderen na correctie voor graviditeit en het bezoek aan een arts in verband met fertiliteitsproblemen.

Het spuiten van bestrijdingsmiddelen uitsluitend door de fruitteler was geassocieerd met een lange tijd tot zwangerschap, resulterend in een fecundabiliteitsratio van 0.46 (95% betrouwbaarheidsinterval: (95% BI 0.28-0.77). Op vergelijkbare wijze resulteerde een lage spuitsnelheid ($\leq$ 1.5 hectare/uur) in een fecundabiliteitsratio van 0.47 (95% BI 0.29-0.76) en is geassocieerd met het gebruik van oudere spuittechnieken en tractoren zonder cabine. Van deze factoren wordt aangenomen dat ze tot een hoge blootstelling leiden. Dit werd bevestigd door blootstellingsmetingen in het veld. Het effect van een hoge blootstelling was voornamelijk duidelijk als de kinderwens bestond in de periode maart-november (fecundabiliteitsratio 0.42, 95% BI 0.20-0.92). Dit is de periode waarin bestrijdingsmiddelen worden gebruikt. Buiten het spuitseizoen was het effect van een hoge blootstelling afwezig (fecundabiliteitsratio 0.82, 95% BI 0.33-2.02). In de hoog-blootgestelde groep werd 28% van de zwangerschappen voorafgegaan door een bezoek aan een arts in verband met fertiliteitsproblemen. In de lage-blootstellingsgroep was dit 8%. Deze bevindingen wijzen erop dat een nadelig effect van blootstelling aan bestrijdingsmiddelen op de fecundabiliteit waarschijnlijk is.

Hoofdstuk 7 beschrijft het gebruik van de geboorte sekse verhouding (verhouding jongens/meisjes) als een indicator voor reproduktieve risico's geassocieerd met blootstelling aan bestrijdingsmiddelen. De hypothese dat de sekse verhouding van de mens bij geboorte mogelijk wordt beïnvloed door factoren in het milieu of beroep is niet nieuw, maar het bewijs ervoor is zwak.

De onderliggende mechanismen zijn nog niet opgehelderd. Effecten op chromosomaal niveau, resulterend in foetale dood voor de mannelijke foetus is gesuggereerd. Ander bewijs wijst in de richting van een mogelijke invloed van hormoonspiegels van de ouders ten tijde van de conceptie. Verschillende grotere studies naar afwijkingen in sekse verhouding gaven inconsistente resultaten te zien. Sommige andere studies hadden de tekortkoming van een beperkte populatieomvang, een slechte schatting van de individuele blootstelling of een slechte correctie voor de vele potentiële verstorende variabelen.

Een voordeel van de geboorte sekse verhouding als een model voor mogelijke gezondheidseffecten is dat deze in principe niet onderhevig is aan informatie bias en eenvoudig is te verzamelen. Indien gevoelig, kan de geboorte sekse verhouding dienen als waarschuwingsteken voor mogelijke nadelige effecten van blootstelling.

In antwoord op een brief aan de editor over onze studie naar tijd tot zwangerschap, werd de geboorte sekse verhouding bestudeerd in dezelfde populatie. Uit de gevonden resultaten in de fruitteelt concludeerden we dat het nuttig lijkt verder na te gaan of de sekse verhouding is gerelateerd aan bestrijdingsmiddelenblootstelling en of de sekse verhouding en tijd tot zwangerschap onderling samenhangen.

In **Hoofdstuk 8** wordt de algemene discussie gepresenteerd. Voor het uitvoeren van bespuitingen met bestrijdingsmiddelen lijken de blootstellingsgegevens representatief te zijn voor *hard fruit* bij gebruik van een getrokken spuit (*'airblast sprayer'*). Voor andere werkzaamheden in de boomgaard hangt het af van de tijdsduur voordat re-entry plaatsvond. Het is niet zeker of de gegevens in dit opzicht representatief waren voor de fruitteelt.

Captan werd representatief geacht voor de poedervormige fungiciden die in de fruitteelt verspoten worden als *'wettable powder'* met een getrokken spuit. Verschillen in afbraak tussen bestrijdingsmiddelen in de boomgaard kunnen echter groot zijn. Als gevolg hiervan kunnen verschillen in blootstelling tijdens werkzaamheden in het gewas optreden en daarom is aanvullende informatie nodig over de afbraak van andere bestrijdingsmiddelen in de boomgaard. Omdat gegevens voor de mens over opname in het lichaam van bestrijdingsmiddelen meestal ontbreken, is een vergelijking over opname van captan met andere middelen niet mogelijk.

Op grond van de verschillende studies werd geconcludeerd dat gezien het feit dat de dag-tot-dag variatie in blootstelling hoog was, herhaalde waarnemingen noodzakelijk zijn om de individuele blootstelling nauwkeurig te schatten.

Aangezien de factoren die de blootstelling bepalen variëren voor de verschillende blootstellingsroutes en delen van het lichaam (huidblootstelling), bepaalt de blootstellingsmaat die voor een bepaalde studie van belang is welke determinanten het meest relevant zijn.

Omdat de populatie fruittelers kan worden gegroepeerd naar potentiële externe blootstelling, kunnen deze blootstellingsgegevens worden gebruikt voor beheersstrategieën om de blootstelling te reduceren en in epidemiologische studies.

Dermale absorptie via enkels en nek vond plaats zonder duidelijke bijdrage van de luchtwegblootstelling. Daarom is in een situatie waar de huid als blootstellingsroute overheerst en de luchtwegroute van ondergeschikt belang is, een grenswaarde voor beroepsmatige blootstelling, die is gebaseerd op de blootstelling van de luchtwegen, niet het juiste uitgangspunt is om een 'huidindicatie' af te leiden.

Aangezien niet-gemeten bronnen van blootstelling de resultaten van epidemiologische studies kunnen vertekenen, is meer aandacht nodig voor de bijdrage van blootstelling in de woonomgeving en voor de niet-werkgerelateerde blootstelling.

Omdat er niet veel epidemiologische studies zijn uitgevoerd naar de effecten van bestrijdingsmiddelen op de reproduktie, kunnen geen harde conclusies worden getrokken over de bestrijdingsmiddelen en andere gerelateerde factoren die verantwoordelijk zijn voor de waargenomen effecten op tijd tot zwangerschap. Deze bevindingen dienen verder onderzocht te worden. De validiteit van de waargenomen effecten op de reproduktie en de onderliggende mechanismen dienen opgehelderd te worden. Het gebruik van de sekse verhouding als effectmaat is mogelijk van belang, maar het wetenschappelijk bewijs is zwak en deze maat zal eerst nader dienen te worden verkend. Zowel tijd tot zwangerschap als sekse verhouding kunnen mogelijk gebruikt worden als eenvoudige en gevoelige blootstellingsmaten in epidemiologische studies om te dienen als waarschuwingsteken voor potentiële nadelige effecten van blootstelling aan bestrijdingsmiddelen. Aangezien zowel de man als de vrouw mogelijk zijn blootgesteld, moet in toekomstige studies meer aandacht worden besteed aan het karakteriseren van de individuele blootstelling aan bestrijdingsmiddelen.

De aanwijzing dat de huidige blootstelling in de fruitteelt mogelijk een effect heeft op de reproduktie, rechtvaardigt dat er meer aandacht wordt besteed aan de mogelijke effecten van blootstelling aan bestrijdingsmiddelen op de reproduktie en aan een reductie van de blootstelling.

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Curriculum Vitae

Johan Sytse de Cock werd geboren op 29 maart 1961 in Geldrop. In 1979, na het behalen van het VWO diploma aan het Comenius College te Hilversum, begon hij zijn studie milieuhygiëne in Wageningen met als specialisatie arbeidshygiëne. Het accent lag op de stofblootstelling en longfunctieveranderingen van werknemers werkzaam bij de overslag van massagoederen te Rotterdam. Op het Coronel laboratorium, faculteit geneeskunde, van de universiteit van Amsterdam hield hij zich daarna bezig met de ontwikkeling van een meetmethode om methyl-ethyl-keton aan te tonen in de urine van beroepsmatig aan oplosmiddelen blootgestelde werknemers. Een stage aan het Hygiënisch Instituut van de universiteit van Aarhus in Denemarken was vervolgens gericht op het testen van een meetmethode om neuropsychologische veranderingen aan te kunnen tonen als gevolg van beroepsmatige blootstelling aan oplosmiddelen. Na het behalen van zijn doctoraal examen in januari 1988 kreeg hij een aanstelling als onderzoeker bij de Landbouwuniversiteit in Wageningen. Allereerst werkte hij aan het project 'Inhoud en organisatie van bedrijfsgezondheidszorg in kleine bedrijven in de melkveehouderij en de loonwerk sector'. Vervolgens begon hij als assistent in opleiding aan het promotieonderzoek 'Blootstelling van fruittelers aan bestrijdingsmiddelen'. Vanaf februari 1995 is hij voor een jaar als 'research fellow' verbonden aan de 'Environmental Epidemiology Unit' van de 'London School of Hygiene and Tropical Medicine', in London. Naast het werk speelt hij viool in diverse orkesten en ensembles.