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Assessment of Inhalation Exposure to Amitraz among Pesticide Sprayers in Zangiabad, Iran

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Abstract: Orchard operations involve heavy use of pesticides to control pests and the potential for exposure of sprayers is high. When high pressure equipments for pesticide application are used, the potential for respiratory exposure is increased. The aim of this study was to assess the amitraz residue level in the individual breathing zone of the sprayers. Individual air samples of 70 amitraz sprayers have been collected using modified fitted with impinger acetonitrile as liquid sorbent. A sample size of 480 liters of air was collected in each case. The mean concentration of amitraz and its metabolite in the breathing area during the application were 11.51 and 1.35 μg/m^3, respectively.

Key words: Occupationally Exposure · Amitraz · Orchard Operation · Pistachio · Iran

INTRODUCTION

Amitraz (*N*-(2,4-dimethylphenyl)-*N*'-[(2,4-dimethylphenyl)-imino]methyl-*N*-methylin ethanimidamide) is a member of formamidine class chemical family [1, 2]. It is a non-systemic acaricide and insecticide, whereby the contact and respiratory actions is used to control insects, ticks and mites [3, 4]. Through a series of intermediate compounds, amitraz hydrolyses to form an environmentally stable compound called 2,4-dimethylaniline (2,4-DMA) [3]. The degradation of amitraz to stabilize aniline products may significantly contribute to the environmental and health risks involved in the application and use of this particular pesticide [5]. More importantly, 2,4-dimethylaniline is also toxic, with an acute oral LD50 of 467 mg/kg for rats, which is almost half that of the parent pesticide [6].

Iran is the largest producer of pistachio in the world, accounting for about two-thirds of the global planted areas and slightly more than one-half of the world's production in the recent years [7]. About 13 different pests and diseases have been found to attack pistachio and 1,800 tonnes of pesticides are used for this particular crop annually [8]. Like many other developing countries, Iran has promoted the use of pesticides to expand agricultural land and increase output per acre. Over 27,000 tonnes of pesticides were used in the Islamic Republic of Iran in 2000/01 and the country spent US$125 million on pesticide imports in 2002 [8].

The potential risk of exposure to pesticide residues in working environments is high [9]. For airborne contamination measurements, close attention must be paid to the breathing zone [10]. Pesticides may be inhaled in efficient dose to cause serious damage to respiratory tract or to be absorbed through the lungs and into the bloodstream. It is crucial to note that the hazard of poisoning from respiratory exposure is great because of the rapid and complete absorption of pesticides through lung tissues. Lungs may be exposed to pesticides by inhalation of powders, airborne droplets or vapours. The hazard from inhalation of pesticide spray droplets is fairly low when diluted sprays are applied with conventional low pressure application equipment. However, when high pressure equipments for pesticide application are used, the potential for respiratory exposure is increased. Some non-fumigant pesticides are toxic to pests as liquid or solid formulations, but they also give off vapours which could be toxic to applicators [11].

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In addition, orchard operations involve heavy use of pesticides in controlling pests and the potential for workers to be exposed to them is high. Safety measures for applicators are still very poor. Many of them do not strictly follow the manufacturer's directions in using the formulations [9]. Respiratory exposure cannot be completely separated from oral or dermal exposure in the sense that some materials which are retained on the mucous membranes of the upper respiratory system will be absorbed through these membranes or swallowed and made available for absorption by the gastrointestinal tract. However, the error is on the side of greater safety if all inhaled material is assumed to represent respiratory exposure, since most, if not all, materials are absorbed more rapidly and more completely through the lungs than through the skin and, for this and perhaps other reasons, are more toxic by the respiratory route [12].

Amitraz replaced zolon for pistachio pest control in Iran about 15 years ago and since then, this particular pesticide has been used in Iran, with approximaly several tonnes of use per year. During that period, amitraz was not regulated and agronomists and farmers had little knowledge about the hazardous risk of using this particular pesticide. In Iran amitraz is available under the proprietry name Mitac with 20% emulsifable amitraz concentrate. Although information on the presence of pesticides in the atmosphere is available [13, 14], data related to determination of pesticides in orchards is still limited, while reports on the assessment of amitraz in pistachio orchards is not found in the literature. The aim of this study was to assess the amitraz residue and metabdite level in the individual breathing zone of the sprayers.

METHODOLOGY

Study Area: Zangiabad is a small city, located 20 km to the north of Kerman, which is the capital of Kerman province. This city is located in an arid desert area with an average annual rainfall of 135mm. The locale is also well-known for growing pistachio nuts. The city is located on a flat plain with an area of 10 km^2 with 5280 hectares of pistachio farms. Amitraz is the most frequently used pesticide in the Zangiabad area for pistachio pest control. The exposure to amitraz may be of occupational origin or strongly related to environmental contamination.

Study Location: This cross-sectional study was carried out in the pistachio orchards in Zangiabad area. The data collection was carried out from April to September 2008 according to the expected frequency and duration of amitraz spraying in the study area. Mature pistachio trees are planted in 6.00-m rows with 3.00-m tree spacing. Individual trees have a height of about 2.50-m and 1.50 m width.The applicators used tractor with high pressure application equipment and a nozzle operating at a flow rate about 15 L per minute for spraying of amitraz. The volume sprayed was 4000 L in each case, corresponding approximately to a dose of 1.5 L per hectar amitraz. The spray liquid was prepared by dispersing 4 L of amitraz 20 % EC in a tank containing 4000 L of water.

Air Sampling: Individual samples of amitraz were collected using liquid sorbent and modified fritted impinger in order to determine the air amitraz concentration. The SKC impinger with a fritted nozzle was modified. The head and stem of impinger was adapted to a 250 mL round bottom flask. The modified impinger, with a mini pump air sampler was used for air sampling. Based on Briand *et al.* [15], the airflow was 2 litres/minute of air sampling. The impinger was filled with 60 mL acetonitrile. Acetonitrile was handled carefully, because it can be a hazard ous when the impinger is mounted on a worker. In order to protect the pump from splashed or spilled impinger liquid, a standard impinger which served as a trap was installed between the impinger and the pump. The sampling device containing the modified impinger, trap and mini pump air sampler was portable. This device was fitted to each sprayer (in front of the chest) with two belts. One belt was put around the neck while the other was securely placed behind the chest. For the comfort and safety of the participants, the sampling equipment was attached to the sprayer so that it would not interfere with their performance or safety.

After sampling, the stopper on the impingers was tightly sealed with parafilm to prevent leakage while travailing to the laboratory. As soon as they were returned to the laboratory from the field, all the samples held in a cold box were stored in the dark at 4 °C. With each batch of ten samples, one midget impinger containing the same volume of acetonitrile, prepared from the same stock as that used for the sample collection, was submitted as the blank. This impinger was subjected to exactly the same handling as the samples except that no air was drawn.

The air samples were collected only during the active spraying phase of each simulation. The pumps were calibrated in the laboratory before going for field operation. A population group of 70 amitraz sprayers

was studied. The origin of the exposure was the use of amitraz on pistachio trees. The sprayers was not permitted to participate in any activity other than spraying. The inlet of impingers was kept in the breathing zone of the applicator and was operated for a period of four hours. A sample size of 480 litres of air was collected. The air was sampled at a flow rate of 2 litres per minute for four hours. In order to prevent the breakthrough of amitraz, the impingers were changed after 2 hours during the field test. The flow rate of the personal sampler pump was checked at the beginning and the end of the exposure period with an SKC calibrator. Pesticide sprayers did not handle concentrated amitraz, but they only handled diluted pesticide which was ready to spray. After air sampling, some protective equipment such as mask and gloves were given to each pesticide operator as an educational health activity to encourage them to use the protective equipment.

Air Samples Extraction Procedure: After sampling, a solvent concentration step was made. At first, the stem of the impingers was rinsed with 2 mL of acetonitrile in the midget impinger flask in the laboratory and this was repeated. The round-bottom flask was attached to a rotary evaporator and the sample was evaporated to around 3.0 mL at 50°C. The sample solution for each impinger was transferred into a separate 6.0-mL glass tube with a Teflon cap and 1.5 mL of acetonitrile was used to wash each impinger. This process was repeated and combined with an appropriate sample solution. After that, the solvent was removed under a soft stream of nitrogen gas for five minutes without heating. The evaporation process was stopped when 1.0 ml of solution was left. A 20-µl volume of internal standard solution thymol (500 ng/ mL in acetonitrile) was added into the extract and the cap of glass tubes were then kept tight and wrapped with an aluminium foil and these were shipped out for analysis immediately. Finally, quantification and confirmation of the results were made using a gas chromatography–mass spectrometer (GC–MS).

GC-MS Analysis: The concentrated stock solution of 1 µg per mL was prepared by diluting pure amitraz and 2,4-dimethylaniline in acetonitrile. The extract was analyzed using the GC–MS. A 1 microliter aliquot of the sample solution was injected into the gas chromatograph. The syringe was cleaned with pure acetonitrile and dried thoroughly between injections. The syringe was then ready to take up the sample for injection. The injection was repeated for each sample. The peak area was measured by the area under the resulting peak and compared with the areas obtained from the injection of standards to prepare calibration curve, as discussed below.

GC-MS Apparatus and Conditions: The analysis was carried out on a GC system coupled with quadrupole mass spectrometer (GCMS-QP5050, Shimadzu Corporation, Japan). The compounds were separated on the ZB-Multiresidue-1 capillary column (Phenomenex, USA, 30m×0.25mm i.d. × 0.25µm film thickness). The injection temperature, GC–MS interface and ion source temperatures were 280, 230 and 230°C, respectively. Meanwhile, the GC oven temperature program utilized an initial temperature of 100 °C and an initial holding time of 5 min and the temperature was increased from 20 °C/min to 136 °C, at which it was held for 2 min and was then increased from 20 °C/min to 300 °C and held for 5 min. Helum was used as carrier gas with a linear speed of 25 cm/s. Amitraz and its metabolite 2,4-dimethylaniline were analyzed in selected full scan mode. The ionizing energy was 70 eV. A 1 µL aliquot of each extract was injected into gas chromatograph. The injection was splitless and the mass spectrometer was calibrated weekly.

Calibration Curves: An eight-point standard calibration curve was made using the analysis of amitraz and 2,4-dimethylaniline. Standard solutions of both analytes were prepared by dissolving the above compounds in acetonitrile to yield the final concentrations of 50, 250, 500, 1000, 2000, 4000, 8000 and 10000 ng/mL. Thymol (500 ng/mL) was used as internal analytical standard. Addition of only acetonitrile (C=0) was used as control. Meanwhile, the peak area ratio (PAR) was obtained from the GC-MS analysis of each compound at different concentrations (ng/mL). After that, the calibration curves were constructed by plotting with the peak area ratio of the analytes and IS on the Y-axis and the concentration on the X-axis.

Calculation: The analyte concentrations for samples were obtained from the calibration curve in terms of micrograms of amitraz per sample. The air concentrations were calculated using the following formulae:

$$\mu g/m^3 = \frac{(\text{micrograms of amitraz per sample}) * (1000)}{(\text{liters of air sampled})}$$

Recovery Efficiency: The extraction recovery was determined by comparing the peak area ratios of amitraz and 2,4-dimethylaniline with the IS of the extracted samples with the peak-area ratios obtained from a direct injection of a standard solution containing the same concentration of amitraz, or its metabolite and the IS (500 ng). In order to determine recovery, three impingers were spiked with analyts to yield 0.05, 0.5 and 5.0 μg/mL concentrations. Amitraz and 2,4-dimethylaniline were diluted in acetonitrile and then extracted according to the same procedure as previously described. Seven replicates were made at each fortification to calculate the mean and standard deviation for recovery. At the same time, a parallel blank was also prepared except that no sample was added to it. The recovery efficiency was calculated using the following equation [16]:

$$\text{Recovery}\% = \frac{(OC_{extract} / IS_{extract})}{(OC_{spike} / IS_{spike})} \times 100\%$$

Where;

***OC* extract =** The peak area for the organic compound (OC) in the extract,

***IS* extract =** The peak area for the internal analytical standard in the same extract,

***OC* spike =** The peak area for the OC in the spike solution and

***IS* spike =** The peak area for the internal analytical standard in the same spike solution.

RESULTS

The amitraz cover spray was applied by sprayers who were moving around the trees, directing spray into foliage and ensuring it was wetted. A single medium-sized tree was sprayed in around 1 min. During spraying, there was visible overspray which was carried by the wind several metres from the point of application. Splashes of the spray from foliage also contributed to the overspray. With respect to the protective equipments used during pesticide application, none of the sprayers in Zangiabad zone used the protective equipments that normally are required as safety devices. Nobody used gloves, masks, plastic cover, boots, apron and waterproof garment.

The average extraction efficiency for seven impingers spiked at the target concentration was 97.3 % and 97.9 % for amitraz and its metabolite, respectively. The average recovery values obtained were at least 95.2 % and as such, no recovery correction factor was used in the determination of the true values. Amitraz and its metabolite were detected in all inhalation air samples of sprayers. The arithmetic mean (AM) and standard deviation (SD) of the concentrations of the target chemicals in the air samples collected are given in Table 1. Amitraz and 2,4-dimethylaniline concentrations in the air samples are expressed as μg analyte per cubic meter of the air sampled (μg/m^3).

The mean concentrations of amitraz and its metabolite in the breathing area during the application were 11.51 and 1.35 μg/m^3, respectively. The average retention time was 22.65 min and 7.11 min for amitraz and 2,4-dimethylaniline, respectively. The following chromatogram (Figure 1) is from injection of 1000 ng/mL amitraz and 1000 ng/mL 2,4-dimethylaniline standards equivalent to 2.08 μg/m^3 of a 480-L air sample for both analytes.

Figure 2 illustrates a chromatogram of a breathing air sample from an amitraz applicator. The concentrations of amitraz and its metabolite in this particular sample were 10.8 and 1.26 μg/m^3, respectively. The retention time in this chromatogram was 22.66 min for amitraz and this was 7.11 min for its metabolite.

In order to evaluate the health hazard involved while working with amitraz, the data of the airborne concentration of this chemical was compared with the toxicological limits. Assuming 5 m^3 as the respiration rate

Table 1: Inhalation Exposure of Amitraz

Parameter	n	Minimum	Maximum	Mean	Std.Deviation
Amitraz (μg/m^3)	70	8.89	14.06	11.5137	1.33070
Metabolite (μg/m^3)	70	.89	1.82	1.3547	.25521

Table 2: Oral ADI, NOEL and Computed Inhalation Intake values for amitraz

Parameter	Oral ADI (mg/kg/d)	Oral ADI (mg/d)	Oral NOEL (mg/kg/d)	Oral NOEL (mg/d)	Computed Inhalation Intake (mg/d)
Amitraz	0.003 (human)		0.25 (dog)		
Adult (70 kg body weight)		0.21		17.5	0.06

(Adaptation: EXTOXNET, 1995; U.S.EPA, 1996)

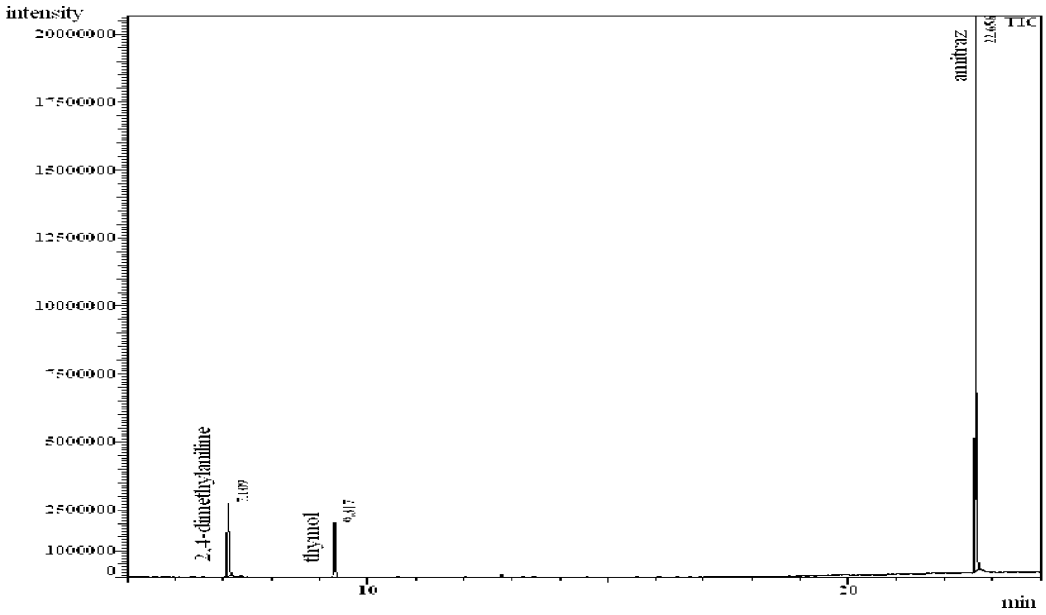

Fig. 1: Chromatogram of Standard Solutions of Amitraz and 2,4-dimethylaniline in an Air Sample

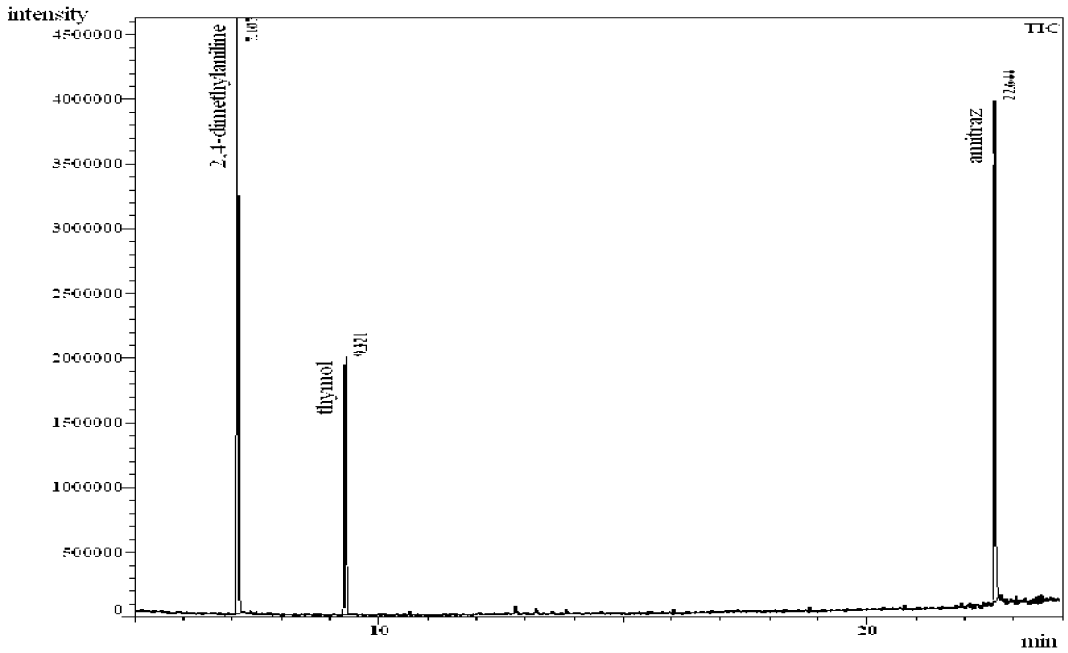

Fig. 2: Typical Chromatogram of the Inhalation Air Sample of an Amitraz Applicator, Zangiabad Area, Iran, 2008

for the average adult per four-hours working [17, 18] and using the concentration of 11.51 µg/m^3 (Table 1), the inhalational intake per working day is equivalent to 57.55 µg/d or 0.057 mg/d. Hence, with the inhalation uptake assumed to be 100 % of the applied dose [19], the daily inhalation exposure for amitraz sprayers would be 0.057 mg.

DISCUSSIONS

The production of pistachio in orchards is associated with specific pest control problems that result in numerous applications of plant protection products. Pistachio trees require intensive care and therefore, sprayers and agricultural workers are frequently exposed

to pesticides in the orchard atmosphere. Methods for the determination of amitraz in air have not been previously described. A general design criterion for a personal sampling device is that it should be small and compact so that the normal daffy functions and jobs can be accomplished with little or no interference from this sampling device [20]. Low-volume samplers which are generally used for personal monitoring are portable, battery operated, relatively quiet and easy to use. Flow rates of 0.5–1.5 L/min are typically recommended for pesticides [21]. In the present study, the applied airflow was 2 L / min as compared to 1.5 L / min to ensure the collection of all the atmospheric phases of amitraz.

Different air sampling methods reported for pesticide determination involve the use of liquid or solid adsorbents and filters [22]. Most field comparisons found that impinger and bubbler methods gave higher results compared to solvent-free methods [23]. Midget impingers or bubblers collect pesticides as aerosols or vapours, but are not well suited to personal sampling since they are cumbersome, breakable and the liquid medium frequently spills during normal work movements. Filters trap aerosols but they do not retain pesticide vapours. Solid sorbents retain pesticide vapours but they may not efficiently collect or trap aerosol forms [20]. Based on these limitations, an air collection method using an impinger which is connected to personal samplers was applied for assessing potential inhalation exposure to amitraz in the present study.

To design the air sampler, optimising the volatilisation was the first step, whereas the shape of the impinger was important for minimizing the sample loss caused by volatilization which could occur during extended sampling periods. Thus, a round flask of 250 mL was chosen for impinger. In the same way, Durham and Wolfe [12] stated a method for sampling the air using the modified impinger and 500-mL Pyrex glass ball. The second step was to choose a proper solvent to be used in the impinger. The most suitable medium for a particular investigation is dependent on the chemicals being studied. The medium should entrap a high percentage of the chemical passing through it and allow the elution of a high percentage of the entrapped chemical for analysis. The chemical should be recovered without any conversion to other reaction products and the medium should not produce a significant restriction of airflow [24]. Since early 1970s, ethylene glycol has been used as a standard media for collecting pesticides in air [15]. Cyclohexane has also been used in impinger for air collection [26]. Amitraz is unstable in pure methanol but it is stable in acetonitrile [26]. For this reason, acetonitrile was chosen as a suitable solvent for air sampling of amitraz. The sampling equipment was attached to the amitraz applicators so that it would not interfere with their work performance or safety.

Some studies have shown that both the type of the collection liquid and the volume affect the collection efficiency. A higher level of liquid means there is more time between bubble formation at the fritted tip and bubble bursting at the surface of the liquid and, thus, more time for particles to diffuse from the air inside the bubbles into the liquid [27]. For this reason, impingers were filled with 60 mL acetonitrile in this study. The results of a study by Haraguchi *et al.* [14] showed that many pesticides exist in a gaseous state rather than in a solid state in air.

In order to evaluate the health hazard involved while working with toxic compounds such as pesticides, it is important to assess the amount of exposure workers undergo while operating the pesticides. After determining the concentration of a pesticide in the air, the respiratory exposure of an exposed person can be calculated using an assumed tidal volume and respiratory rate [12]. A comparison with the ADI was also calculated although the ADI (mg/kg body wt/day) refers to oral adsorption. Table 2 summarizes Acceptable Daily Intake (ADI) and No Observed Adverse Effect estimates (NOEL) and computed inhalation intake values for amitraz.

CONCLUSION

The oral No Observed Effect Level (NOEL) in a 70 kg adult is 17.5 mg/d. Since the mean concentration of amitraz in pesticide sprayers was 11.51 $\mu g/m^3$ and the inhalation intake in this study was 0.06 mg/d, is lower than the acceptable daily intake for adults of 0.21 mg/d, it appears that no serious and urgent risk is to be expected and acute poisoning will not occur due to amitraz exposure. However, this chemical agent and its metabolites may cause chronic adverse health effects after a long period of continuous exposure.

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