

**Volume 200**

# Reviews of Environmental Contamination and Toxicology

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Reviews of  
Environmental Contamination  
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VOLUME 200

# Reviews of Environmental Contamination and Toxicology

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# Foreword

International concern in scientific, industrial, and governmental communities over traces of xenobiotics in foods and in both abiotic and biotic environments has justified the present triumvirate of specialized publications in this field: comprehensive reviews, rapidly published research papers and progress reports, and archival documentations. These three international publications are integrated and scheduled to provide the coherency essential for nonduplicative and current progress in a field as dynamic and complex as environmental contamination and toxicology. This series is reserved exclusively for the diversified literature on “toxic” chemicals in our food, our feeds, our homes, recreational and working surroundings, our domestic animals, our wildlife and ourselves. Tremendous efforts worldwide have been mobilized to evaluate the nature, presence, magnitude, fate, and toxicology of the chemicals loosed upon the earth. Among the sequelae of this broad new emphasis is an undeniable need for an articulated set of authoritative publications, where one can find the latest important world literature produced by these emerging areas of science together with documentation of pertinent ancillary legislation.

Research directors and legislative or administrative advisers do not have the time to scan the escalating number of technical publications that may contain articles important to current responsibility. Rather, these individuals need the background provided by detailed reviews and the assurance that the latest information is made available to them, all with minimal literature searching. Similarly, the scientist assigned or attracted to a new problem is required to glean all literature pertinent to the task, to publish new developments or important new experimental details quickly, to inform others of findings that might alter their own efforts, and eventually to publish all his/her supporting data and conclusions for archival purposes.

In the fields of environmental contamination and toxicology, the sum of these concerns and responsibilities is decisively addressed by the uniform, encompassing, and timely publication format of the Springer triumvirate:

*Reviews of Environmental Contamination and Toxicology* [Vol. 1 through 97 (1962–1986) as Residue Reviews] for detailed review articles concerned with any aspects of chemical contaminants, including pesticides, in the total environment with toxicological considerations and consequences.

*Bulletin of Environmental Contamination and Toxicology* (Vol. 1 in 1966) for rapid publication of short reports of significant advances and discoveries in the fields of air, soil, water, and food contamination and pollution as well as methodology and other disciplines concerned with the introduction, presence, and effects of toxicants in the total environment.

*Archives of Environmental Contamination and Toxicology* (Vol. 1 in 1973) for important complete articles emphasizing and describing original experimental or theoretical research work pertaining to the scientific aspects of chemical contaminants in the environment.

Manuscripts for Reviews and the Archives are in identical formats and are peer reviewed by scientists in the field for adequacy and value; manuscripts for the *Bulletin* are also reviewed, but are published by photo-offset from camera-ready copy to provide the latest results with minimum delay. The individual editors of these three publications comprise the joint Coordinating Board of Editors with referral within the Board of manuscripts submitted to one publication but deemed by major emphasis or length more suitable for one of the others.

Coordinating Board of Editors

# Preface

The role of Reviews is to publish detailed scientific review articles on all aspects of environmental contamination and associated toxicological consequences. Such articles facilitate the often-complex task of accessing and interpreting cogent scientific data within the confines of one or more closely related research fields.

In the nearly 50 years since *Reviews of Environmental Contamination and Toxicology* (formerly *Residue Reviews*) was first published, the number, scope and complexity of environmental pollution incidents have grown unabated. During this entire period, the emphasis has been on publishing articles that address the presence and toxicity of environmental contaminants. New research is published each year on a myriad of environmental pollution issues facing peoples worldwide. This fact, and the routine discovery and reporting of new environmental contamination cases, creates an increasingly important function for *Reviews*.

The staggering volume of scientific literature demands remedy by which data can be synthesized and made available to readers in an abridged form. Reviews addresses this need and provides detailed reviews worldwide to key scientists and science or policy administrators, whether employed by government, universities or the private sector.

There is a panoply of environmental issues and concerns on which many scientists have focused their research in past years. The scope of this list is quite broad, encompassing environmental events globally that affect marine and terrestrial ecosystems; biotic and abiotic environments; impacts on plants, humans and wildlife; and pollutants, both chemical and radioactive; as well as the ravages of environmental disease in virtually all environmental media (soil, water, air). New or enhanced safety and environmental concerns have emerged in the last decade to be added to incidents covered by the media, studied by scientists, and addressed by governmental and private institutions. Among these are events so striking that they are creating a paradigm shift. Two in particular are at the center of ever-increasing media as well as scientific attention: bioterrorism and global warming. Unfortunately, these very worrisome issues are now super-imposed on the already extensive list of ongoing environmental challenges.

The ultimate role of publishing scientific research is to enhance understanding of the environment in ways that allow the public to be better informed. The term “informed public” as used by Thomas Jefferson in the age of enlightenment

conveyed the thought of soundness and good judgment. In the modern sense, being “well informed” has the narrower meaning of having access to sufficient information. Because the public still gets most of its information on science and technology from TV news and reports, the role for scientists as interpreters and brokers of scientific information to the public will grow rather than diminish. Environmentalism is the newest global political force, resulting in the emergence of multi-national consortia to control pollution and the evolution of the environmental ethic. Will the new politics of the 21st century involve a consortium of technologists and environmentalists, or a progressive confrontation? These matters are of genuine concern to governmental agencies and legislative bodies around the world.

For those who make the decisions about how our planet is managed, there is an ongoing need for continual surveillance and intelligent controls, to avoid endangering the environment, public health, and wildlife. Ensuring safety-in-use of the many chemicals involved in our highly industrialized culture is a dynamic challenge, for the old, established materials are continually being displaced by newly developed molecules more acceptable to federal and state regulatory agencies, public health officials, and environmentalists.

*Reviews* publishes synoptic articles designed to treat the presence, fate, and, if possible, the safety of xenobiotics in any segment of the environment. These reviews can either be general or specific, but properly lie in the domains of analytical chemistry and its methodology, biochemistry, human and animal medicine, legislation, pharmacology, physiology, toxicology and regulation. Certain affairs in food technology concerned specifically with pesticide and other food-additive problems may also be appropriate.

Because manuscripts are published in the order in which they are received in final form, it may seem that some important aspects have been neglected at times. However, these apparent omissions are recognized, and pertinent manuscripts are likely in preparation or planned. The field is so very large and the interests in it are so varied that the Editor and the Editorial Board earnestly solicit authors and suggestions of underrepresented topics to make this international book series yet more useful and worthwhile.

Justification for the preparation of any review for this book series is that it deals with some aspect of the many real problems arising from the presence of foreign chemicals in our surroundings. Thus, manuscripts may encompass case studies from any country. Food additives, including pesticides, or their metabolites that may persist into human food and animal feeds are within this scope. Additionally, chemical contamination in any manner of air, water, soil, or plant or animal life is within these objectives and their purview.

Manuscripts are often contributed by invitation. However, nominations for new topics or topics in areas that are rapidly advancing are welcome. Preliminary communication with the Editor is recommended before volunteered review manuscripts are submitted.



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# Illnesses Associated with Chloropicrin Use in California Agriculture, 1992–2003

Michel Oriel, Susan Edmiston, Sheryl Beauvais, Terrell Barry,  
and Michael O’Malley

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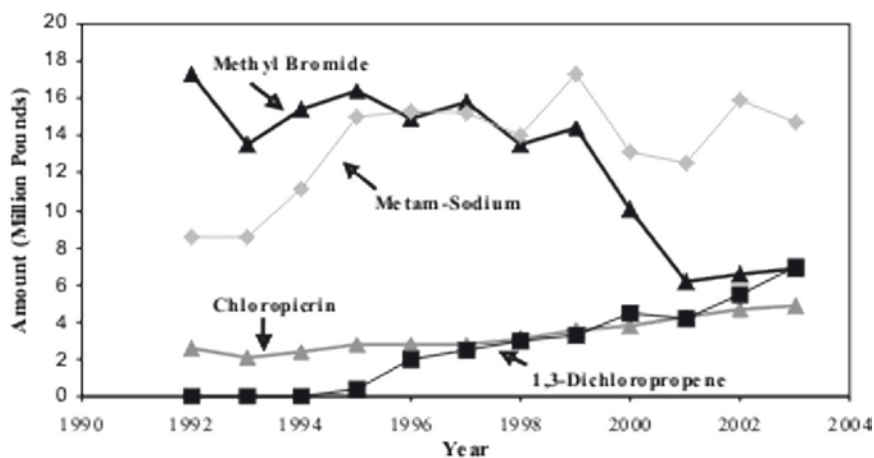
## 1 Introduction

Because methyl bromide use is limited by international treaty (Albritton et al. 1998; U.S. EPA 2004), use of metam-sodium, chloropicrin, and other fumigants have increased (Fig. 1), and have been accompanied by multiple community illness episodes (O’Malley et al. 2004a, b, 2005). The purpose of this article is to review the California experience with the use of chloropicrin as an active ingredient in agricultural fumigations between the years 1992 and 2003.

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**Fig. 1** Agricultural use, in California, of methyl bromide and alternative fumigants, 1992–2003. Fumigant use data for the years 1992–2003 were obtained from the California Department of Pesticide Regulation’s Pesticide Information Portal (CalPIP). Amounts reported reflect the combined total pounds applied annually for all agricultural-related crops sites

## 2 Chloropicrin: History and Essential Background

### 2.1 History

Chloropicrin was initially synthesized in 1848 by addition of bleaching powder to picric acid, and was initially patented in 1908 for use as an insecticide in the fumigation of stored grains and for nematode soil treatments.

During WW I, chloropicrin was used as a tear gas and was the subject of several experimental studies with small numbers of volunteer subjects. In 1921, Fries and West reported on the ocular dose response for four subjects: the time to involuntary eye closure for chloropicrin concentrations was between 2 and 38 sec, at concentrations between 2 and 20 ppm (Fries and West 1921). Concentrations below 1–2 ppm produced “considerable blinking” but not eye closure. Flury and Zernik (1931) summarized the results of German studies (original citation by Gildemeister and Heubner 1920) on chloropicrin. These studies demonstrated that chloropicrin concentrations of 0.3–3.7 ppm produce involuntary eye closure within 3–30 sec. Concentrations of 15 ppm could not be tolerated by unhabituated subjects for longer than 1 min (Flury and Zernik 1931). In addition to intense eye irritation, wartime exposure to chloropicrin was associated with coughing and severe gastrointestinal effects, which included persistent nausea, vomiting, colic, and diarrhea (Fries and West 1921; Prentiss 1937).

## 2.2 *Registered Products*

Chloropicrin is classified as a Toxicity Category I substance and is a restricted use pesticide. It is labeled with the signal word “Danger” (Meister 2003). Applications occur at least 4 d prior to planting, with liquid fumigant injected into soil approximately 6–10 in. below the surface. Target pests include soilborne fungi, nematodes, and soil insects (Wilhelm 1996; Meister 2003).

As of May 2006, there were 48 formulations containing chloropicrin as an active ingredient that were actively registered in California (Department of Pesticide Regulation (DPR) 2006). These included 13 formulations with no other active ingredient (concentrations 94–100% chloropicrin), and 11 formulations containing combinations of dichloropropene (concentrations 37.6–82.9%) and chloropicrin (concentrations 15–60%). Although the Montreal Protocol was to have eliminated use of methyl bromide by the end of 2005, there were 24 formulations of chloropicrin extant (concentrations 24–55%) that contained methyl bromide (concentrations 33–80%) as of February 2006. Another use of chloropicrin is as a warning agent in sulfuryl fluoride structural applications, although it is introduced to the structure at the time of fumigation rather than being mixed with the sulfuryl fluoride in the formulation process.

During 2004, chloropicrin was the eighth most widely used pesticide in California (all sites combined) (DPR 2005). In the USA, chloropicrin moved from being the 39th most commonly used pesticide in the agricultural market sector in 1993, to being the 14th most used, in 1999 (Donaldson and Kiely 2002).

## 2.3 *Physical and Chemical Properties*

Chloropicrin is the common name for the chemical trichloronitromethane, or nitrochloroform. It is a colorless to faint-yellow oily liquid with a strong odor that can be described as pungent, sweet, and irritating, or resembling flypaper (Prentiss 1937; Hayes and Lawes 1991). Chloropicrin has a molecular weight of 164.4 D (Daltons), a specific gravity of 1.66 at 20°C, and a density of 1.65 g cm<sup>-3</sup> at 20°C. It has a boiling point of 112°C, melting point of 64°C, and a vapor pressure of 18.3 mmHg at 20°C (Great Lakes Chemical Corporation 2001). It is practically insoluble in water (0.18 g/100 g at 20°C) but is miscible with benzene, absolute alcohol, and carbon disulfide. Decomposition of chloropicrin may release the following toxic gases and vapors: phosgene, chlorine, carbon monoxide, nitrosyl chloride, and oxides of nitrogen (American Conference of Governmental Industrial Hygienists 1996).

## 2.4 *Legal Exposure Limits*

The current threshold limit value (TLV), set for chloropicrin in 1959, is 0.1 ppm for an 8-hr time-weighted average exposure. Documentation for this standard cites

a review by Stokinger (1978), based upon a brief review of animal and human studies on chloropicrin published in 1931 (Flury and Zernik 1931). The 1931 review summarized WWI era human studies as follows:

... concentrations of 0.3 to 0.37 ppm resulted in eye irritation in 3 to 30 sec, depending on individual susceptibility.

A concentration of 15 ppm could not be tolerated longer than 1 min, even by individuals accustomed to chloropicrin.

In 1959, the TLV was reduced to 0.1 ppm. According to Stokinger, this was done “to provide greater protection from eye irritation in all workers and to ensure against potential pulmonary changes”. “It [the 0.1 ppm TLV] is below concentrations detectable by odor or irritation” (Stokinger 1978).

### 3 Chloropicrin: Exposure and Effects

#### 3.1 Volunteer Studies

Experimental studies with pesticides and other industrial products are often controversial, particularly when nonpharmaceuticals (such as pesticides) are intentionally administered to humans, regardless of potential study benefits (Bates et al. 2005; Boxer and Waxman 2005; Kaiser 2003). Controversy surrounded a human volunteer study (Cain 2004) performed on the irritant effects of chloropicrin (Lee and Clark 2005). Notwithstanding, the Cain study demonstrated chloropicrin effects below the current TLV.

Key findings of this study are summarized below:

- The median instantaneous odor detection threshold for all subjects was 700 ppb. The perception of odor diminished with prolonged exposure, while the perception of irritation increased.
- For brief exposures (5–30 sec), eye irritation (or at least “chemesthesis” – detection of exposure by the eye) occurred in 50% of volunteer subjects exposed to 700 ppb of chloropicrin. Of the 62 subjects tested, 10–15% (depending upon the site of exposure) failed to detect 1,200 ppb of chloropicrin (the highest concentrations tested in the study).
- For exposures lasting 20 min, the minimum concentration detectable by 50% of the subjects was 75 ppb. The no-observed-effect-level (NOEL) for this portion of the study was 50 ppb. One-hr exposures to 100 and 150 ppb produced subjective eye irritation in most subjects. Using a standard tenfold uncertainty factor (Extoxnet 2006), an estimated NOEL for a 1-hr exposure to chloropicrin (calculated from the 100 ppb LOEL (lowest-observed-effect-level)) was 10 ppb.
- In the subjects studied (selected for absence of asthma, allergic rhinitis, and other common respiratory conditions), respiratory irritation was not quantifiable for brief exposures above 1 ppm. Similarly, no upper or lower respiratory symptoms

were noted in either the 20 min (75 ppb), or 60 min (100 and 150 ppb) exposures (Cain 2004).

The distinction between chemesthesis and irritation made by Cain is not made by other specialists in sensory irritation. A review by Dalton (2001), for example, uses the terms “chemesthesis” and “irritation” interchangeably. She makes the point that the onset of sensory irritation is highly influenced by subjective factors. In this regard, the subjective experience of a paid volunteer, in an experimental study, probably would be different from the subjective experience of someone accidentally exposed to the same concentration of an irritant in the workplace, or as the result of an unexpected environmental exposure.

## **3.2 Exposure Monitoring Studies**

Exposure monitoring studies of preplant soil treatment using chloropicrin fumigations have been conducted by product manufacturers and by the state of California; worker exposure and/or downwind air concentrations were recorded after these treatments. In addition, though not elaborated here, attempts have been made in a few exposure studies of structural fumigations to either document “warning” concentrations of chloropicrin inside treated structures, or to record levels of post-application chloropicrin residues (Fong 2004).

### **3.2.1 Worker Monitoring**

In 1982, workers were monitored, in the breathing zone, during two preplant soil applications (Maddy et al. 1984a). The first involved applying a mixture of 33% chloropicrin and 67% methyl bromide (300 lb of formulated product/A). The second application employed 75% methyl bromide/25% chloropicrin (275 lb of formulated product/A). The monitored workers included two application rig drivers, two rig “co-pilots” (seated at the back of the rig, and responsible for ensuring that the injectors and other application equipment were operating correctly), and a “shoveler” (responsible for covering the edges of the application tarp with soil) at the second application site only.

Drivers and copilots were exposed to breathing-zone concentrations of methyl bromide (0.4–6.3 ppm), and chloropicrin (from the < 1 ppb limit of detection to 181 ppb). The “shoveler”, monitored for 45 min, had exposure to breathing zone concentrations of 0.7 ppm of methyl bromide and 45 ppb of chloropicrin (Maddy et al. 1984a). Methyl bromide measurements were associated with “break through” in the sampling tubes and may have underestimated the actual concentrations present (Maddy et al. 1984a).

Subsequently, soil fumigation treatments, monitored in August and September, 1983 (Maddy et al. 1984b), involved the same two products (75% methyl bromide/25%

chloropicrin and 67% methyl bromide/33% chloropicrin), but demonstrated markedly higher exposures. Drivers were exposed to breathing-zone methyl bromide concentrations between 3.1 and 38 ppm; chloropicrin exposure ranged between 90 and 1,544 ppb.

Exposure monitoring studies sponsored by the Chloropicrin Manufacturers' Task Force were reported as estimated 8-hr time-weighted-average exposures (Rotandaro 2004). The highest chloropicrin concentrations were found for drivers in the shank-bedded nontarped method (Sites 13–15); the arithmetic mean + standard deviation (SD) concentration was  $255 + 120 \mu\text{g m}^{-3}$  (38 ppb). Concentrations measured outside the cabs for these drivers were higher; the two replicates gave results of  $208 \mu\text{g m}^{-3}$  (31.2 ppb) and  $1,020 \mu\text{g m}^{-3}$  (153 ppb), for a mean of  $614 \mu\text{g m}^{-3}$  (92.1 ppb).

During shank broadcast applications, average exposures to rig drivers were  $118 \mu\text{g m}^{-3}$  (17.7 ppb). Average exposures for soil sealers (workers who follow the applicator, driving a second tractor equipped with soil disc and cultipacker, or similar device) was  $66.6 \mu\text{g m}^{-3}$  (9.9 ppb). Exposures to re-entry workers ("soil shapers", tarp punchers and pipe layers) ranged from 8.84 to  $75.9 \mu\text{g m}^{-3}$  (1.36–11.25 ppb) (Rotandaro 2004).

### 3.2.2 Downwind Monitoring

In 1982 (Maddy and Gibbons 1983), methyl bromide and chloropicrin levels were monitored at three application sites in Southern California, 25 ft downwind from a shallow injection application of methyl bromide and chloropicrin. Resultant residue levels of methyl bromide ranged from below the limit of detection (<22 ppb) to 634 ppb. The peak levels of chloropicrin (in samples collected for 45 min) detected at the three sites were 33, 76, and 106 ppb, at varying intervals after the application. Only limited data were contained in the report about the application mixture and the environmental conditions.

In 1983, Maddy et al. (1984c) monitored chloropicrin and methyl bromide residues for 1 hr 50 ft downwind from two preplant soil applications. The specific mixture applied to the treated field and the details of the environmental conditions were not specified in the report. Methyl bromide concentrations ranged from below the limit of detection (<3ppb) to 814 ppb, and chloropicrin concentrations varied from < 1 ppb to 81 ppb.

In 1996, the Chloropicrin Manufacturers' Task Force submitted monitoring data to the California Department of Pesticide Regulation on preplant soil fumigations conducted during 1995 near Phoenix, AZ. Monitoring was conducted for a 6-hr duration up to 180 ft downwind of the applications, which were conducted using four different methods (Beard et al. 1996). Samplers were typically located at 60-, 120-, and 180-ft downwind from the site of application. The results are summarized below:

1. *Broadcast-untarped application (171lb/A effective broadcast rate)*. Three samplers on the east transect showed 6-hr time-weighted average concentrations of 0.27 ppm, 0.25 ppm, and 0.26 ppm at 60 ft, 120 ft, and 180 ft, respectively.

2. *Bedded-untarped application (86 lb/A effective broadcast rate)*. During the first 6-hr sampling period, the concentrations in the 60-ft and 120-ft samplers on the north transect were 0.26 ppm and 0.18 ppm, respectively. The concentration at the 60-ft west sampler was 0.16 ppm. During the second 6-hr period, following the 6–12-hr application, the north transect showed air concentrations for the 60-, 120-, and 180-ft samplers of 0.22 ppm, 0.15 ppm, and 0.16 ppm, respectively.
3. *Bedded-tarped application (189 lb/A effective broadcast rate)*. During the period 6–12 hr after application, the 60-ft east sampler showed 0.15 ppm and the 60-ft south sampler showed 0.18 ppm. Concentrations exceeding 0.15 ppm were observed for samplers at 60, 120, and 180 ft. During the 24–30-hr sampling period following application, the 60-, 120-, and 180-ft east samplers showed 0.19 ppm, 0.19 ppm, and 0.16 ppm, respectively. The 60-ft south sampler showed 0.19 ppm. During the 30–36 hr following application sampling (night and evening hours), the 60-, 120-, and 180-ft south samplers showed 0.27 ppm, 0.21 ppm, and 0.15 ppm, respectively. For the same application rates bed-tarp applications have much higher flux than does broadcast-tarp.
4. *Broadcast-tarped application (332 lb/A effective broadcast rate)*. Even though the broadcast-tarped application rate (332 lb/A) was 1.75 times higher than the effective broadcast rate of the bed-tarp application (189 lb effective broadcast rate/A), the measured concentrations of chloropicrin were generally lower than those associated with the bedded tarped application. There were several samplers that showed measured concentrations between 0.10 and 0.15 ppm. In studies with methyl bromide, differences in air concentrations and flux between bed-tarp and broadcast-tarp methyl bromide applications were also observed.

### 3.2.3 Flux Measurements

Onsite measurements made during the study (Beard et al. 1996) also allowed for estimation of flux (kilogram of evaporated chloropicrin/A/24 hr); with an estimation of flux, calculation of chloropicrin dispersion at downwind distances greater than those actually monitored (e.g., with the Industrial Source Complex (US EPA OAQPS 1995; O'Malley et al. 2004a) (ISC3 model) was possible. Flux estimates gleaned from the study are summarized in Table 1.

The Chloropicrin Manufacturers' Task Force submitted the results of an occupational exposure study conducted in 2004, in which downwind worker monitoring results were reported at 2 of 27 sites. For site 16, 4.5 A of an 8.67-A field was treated with chloropicrin EC (Emulsifiable Concentrate) at 300 lb/A by surface drip (tarped and bedded) application. During the first 48 hr after application, samples were collected at 4-hr intervals using XAD-4® resin tubes. After 48 hr, the sampling interval duration varied between 9 and 13 hr. For the initial 4-hr sampling interval, the measured concentration of chloropicrin was slightly less than 0.04 ppm (40 ppb). For the second 4-hr interval, the measured concentration was approximately 0.055 ppm (55 ppb). Peak off-site flux (70 µg of chloropicrin/m<sup>2</sup>/sec) was also measured during



**Table 1** Flux associated with chloropicrin applications in the Beard et al. (1996) study

Application type	24 hr flux: (kg/A/24 hr) in the first 3 d after application of chloropicrin using four application types		
	0–24 hr	24–48 hr	48–72 hr
Untarped			
Bedded (86-lb effective broadcast rate/A)	15.7	1.7	0.35
Broadcast (171 lb/A)	29.1	10.8	3.36
Tarped			
Bedded (189-lb effective broadcast rate/A)	26.6	25.2	4.06
Broadcast (332 lb/A)	37.8	30.0	11.5

For a specific application method, flux is generally proportional to the effective broadcast application rate. This allows adjustment of flux estimates for comparison between application methods

the second 4-hr postapplication interval (approximately 24 kg/A/24 hr, comparable to the results of the 1996 study).

For the greenhouse application at site 24, the application rate was 310 lb/treated A and 0.0741 A was treated, representing a total of 22.97 lb of chloropicrin. Sampling just feet outside the treated area showed peak concentrations of approximately 0.085 ppm (85 ppb) during the second 4-hr interval after the start of the application.

## 4 Chloropicrin-Associated Illnesses in California Between 1992 and 2003

During the 12-yr period covered by this report, 28 individual episodes gave rise to 288 definite, probable, or possible health-effects case reports related to drift, during agricultural applications of chloropicrin (Table 2). An additional 30 cases, related to three separate episodes<sup>1</sup>, were related to off-site exposure. Of the 318 drift and off-site movement cases, 306 (96.2%) were part of illness clusters, including nine formally designated priority investigations (individual cases hospitalized 24 hr or longer, or clusters of five or more cases). These included a 1995 episode that affected 16 people in Ventura County, CA, and a 2003 episode that affected 166 people in Kern County, CA.<sup>1</sup>

Most of the reported exposures were nonoccupational in nature (264 cases or 83%) and involved residents living near treated fields. Among the 54 occupational

<sup>1</sup>Information on this case published in the MMWR (Morbidity and Mortality Weekly Report) of August 20, 2004;53(32):740–742 referenced a total of 165 cases; one additional case is included in this report based upon information reviewed since that article was published.

**Table 2** Individual illnesses and number of drift and off-site movement episodes, in California, involving chloropicrin for the years 1992–2003

Illness reporting year	Cases related to drift (post-application offsite movement)			<i>Total for year</i>
	Priority episode or reference number	Cluster illness totals	Noncluster illnesses	
1992	1992–827 (SD 7, cases), 29-MER-92 (6 cases), 51-VEN-92 (11 cases)	24	1	25
1993	51-Tul-83		3 <sup>a</sup>	3
1994	1994–433	3	3	6
1995	43-VEN-95 (16 cases), 1995–2025 (9 cases)	25	1	26
1996		0	1	1
1998	1998–257	7	1	8
1999	1999–354	6	0	6
2000	44-MON-00	9	0	9
2001	2001–795 (2 cases), 2001–8 (2 cases), 22-SJ-01 (10 cases)	14	1	15
2002	3-SLO-02 (5 cases), 69-SLO-02 (5 cases)	10	1	11
2003	7-SJ-03 (12 cases), 36-Ker-03 (166 cases)	178	0	178
<i>Total</i>		276	12	288
	Cases related to off-site movement			
2002	3-SLO-02 (7 cases), 69-SLO-02 (9 cases)	16	0	16
2003	31-ORA-03	14	0	14
<i>Total</i>		30	0	30

Data for the years 1992–2003 were generated on February 23, 2005, by Dr. Louise Mehler, Worker Health and Safety Branch, Pesticide Illness Surveillance Program, Department of Pesticide Regulation

<sup>a</sup> 51-TUL-93 involved an application worker hospitalized for observation

cases, illnesses occurred in emergency responders and were most frequent (21 cases) when incidents produced nonoccupational case reports. Agricultural workers affected included both applicators (14 cases) and field workers (11 cases).

Eye irritation (watery and burning eyes) was reported in 280 cases (88.1%), including 90 (28.3%) with isolated ocular symptoms. Respiratory irritation (nose and throat irritation, chest pain, shortness of breath, or coughing) occurred in 173 (54.4%), but only 10 cases (3.1%) had isolated respiratory symptoms. Systemic symptoms (headache, nausea, and vomiting) occurred in 149 cases (46.9%), but did not occur as an isolated symptom pattern. Skin irritation was only noted in five cases (1.6%), always in conjunction with eye, respiratory or systemic symptoms.

The 173 cases of respiratory symptoms included 141 with cough, shortness of breath, or chest pain, possibly representing lower respiratory irritation. There were 18 instances of aggravation of pre-existing asthma.

#### 4.1 *Illness Clusters and Priority Investigations Resulting from Chloropicrin Drift in California*

There were 14 drift episodes involving clusters of five or more illnesses (including eight formally identified as priority investigations by California Department of Pesticide Regulation) associated with agricultural use of chloropicrin from the years 1992 through 2003; these involved a total of 264 individuals (91.7% of the 288 total cases). There were three additional episodes involving either two or three cases, each.<sup>2</sup> A categorization of the symptoms observed in these incidents is presented in Table 3.

**Table 3** Summary of symptoms reported for chloropicrin-related priority episodes

Priority/Reference number	Cases related to drift							Total
	Eye	Eye only	Respiratory	Respiratory only	Systemic	Systemic only	Skin	
<i>Nonpriority cases</i>	7	2	7		7	2		11
1992–827	7	2	3		2			7
29-MER-92	6	2	4		2			6
51-VEN-92	11	3	7		1			11
51-TUL-93			1	1				1
1994–433	3		3		2			3
1995–2025	9	7	2		1			9
43-VEN-95	16	2	9		13			16
1998–257	7	3	3		1			7
1999–354	5	1	5	1	1			6
44-MON-00	8		5	1	8			9
2001–795	2	1	1				1	2
2001–8	1		2		1			2
22-SJ-01	8		10		10			10
3-SLO-02			3	2	3	2		5
69-SLO-02			1		4			5
7-SJ-03	12		12					12
36-KER-03	164	54	82		84	2	3	166
<i>Total drift cases</i>	266	77	161	7	136	6	4	288
	Cases related to off-site movement							
3-SLO-02			4	1	6	3		7
69-SLO-02			7	2	7	2		9
31-ORA-03	14	13	1					14
<i>Total of all cases</i>	280	90	173	10	149	11	4	318

Data for the years 1992–2003 were generated on February 23, 2005, by Dr. Louise Mehler, Worker Health and Safety Branch, California DPR. Priority investigations are listed by the county and the year in which they occurred. DPR Assigned Priority Number: Priority investigation number assigned by DPR for reference. Symptoms (Individuals usually reported more than one symptom type following an exposure)

<sup>2</sup>One episode was designated as a priority because an individual was hospitalized for greater than 24 hr.

The cluster episodes discussed below have several common elements:

1. a pattern of mixed ocular, respiratory, and systemic symptoms
2. juxtaposition of fumigated fields with either housing or workers present in adjacent fields, typically with buffer zones of 500 ft or less
3. shifts in wind that placed adjacent fields or housing downwind from the site of fumigation
4. low wind speeds or temperature inversions

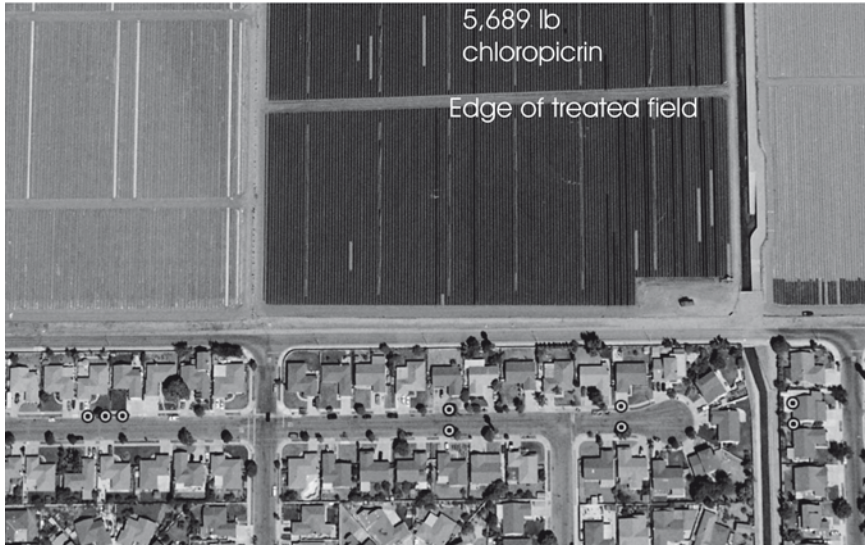
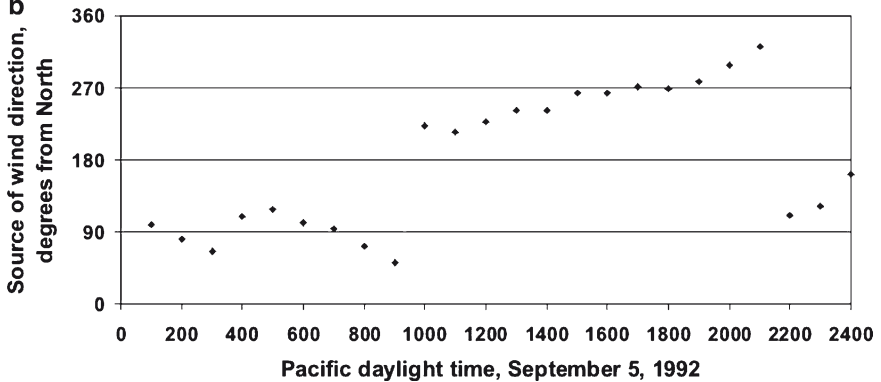
#### **4.1.1 1992 Merced County**

In 1992, a grower in Merced County treated and tarped a 10-A section of preplant strawberries with a fumigant containing 33% chloropicrin and 67% methyl bromide; the applied rate was 323 lb of product/A (106.6 lb of chloropicrin/A). According to the pesticide use report, the grower completed the application about 5:00 p.m. Within approximately 30 min following the completed application (5:00–5:30 p.m.), four residents, living to the east and approximately 100 ft north of the field, began to complain of burning eyes and throat, runny nose, difficulty breathing, coughing, nausea, and vomiting. Two responding police officers were momentarily exposed to vapors when they arrived on the scene, and experienced burning eyes. They immediately moved to a safe location. Emergency responders determined that the chemical would dissipate naturally in a few hours, so the roads were closed for a few hours, and area residents were notified and given the opportunity to evacuate, but none chose to leave. The four nearby residents and two responding police officers sought medical attention for their symptoms.

An examination of the 1-mil tarp found no tears in the seams or elsewhere that could have resulted in escaping fumes. Responding emergency personnel concluded that the problem was probably caused by warm and still air, which allowed the fumigant to build up and remain in the residential area (Irrigation management weather stations located 24 miles south and 27 miles northwest of the treated site reported that, on that day at 5:00–5:30 p.m., the wind speed was 5–7 miles/hr, and blowing from the northwest). No violations were discovered during the investigation.

#### **4.1.2 1992 Ventura County**

A Ventura County agricultural pest control operator (AGPCO) treated 49 A of a preplant strawberry field with a fumigant containing 32.7% chloropicrin and 67% methyl bromide at a rate of 355 lb of product/A (116.1 lb of chloropicrin/A – a total of 5,688.9 lb of chloropicrin), at a distance of 412 ft from the nearest occupied property (Fig. 2a). The AGPCO used a 1-mil tarp and completed the application at 5:30 p.m. At 8:53 p.m. that evening, the local fire department was dispatched when a resident reported a strong odor. Data from the Port Hueneme CIMIS (California

**a****b**

**Fig. 2** (a) 1992 Ventura County. Exposure incident occurred during the evening of September 5, 1992, in the community of Oxnard, with initial symptoms reported at 8:45 p.m. The buffer distance between the south end of the treated section of the field and the affected properties was approximately 400 ft. (b) The CIMIS (The California Irrigation Management Information System) weather station 97 at Port Hueneme, located 3 miles west of the treated field, reported that wind was from the west ( $270^\circ$ ) between 3 and 7 p.m., shifting to northwest ( $315^\circ$ ) between 8 and 10 p.m. The initial report of symptoms was at 8:45 p.m. (2045 in military time, as shown in the graph)

Irrigation Management Information System) weather station, approximately 3 miles west, indicated that wind was from the east at 7 p.m., but veered to the northeast between 8 and 9 p.m. (Fig. 2b).

The fire department crew arrived to find several residents standing and complaining of irritant symptoms. Eleven residents reported nausea, eye irritation, headache,

dizziness, and shortness of breath, symptoms consistent with exposure to chloropicrin. All responded to first aid given by ambulance crews and did not require additional treatment. The county hazardous materials (HAZMAT) unit tested the responding city fire fighters for the presence of methyl bromide, but did not sample for chloropicrin.

The following violations were noted for this case:

1. Prevention of substantial drift to nontarget areas was not adhered to (label violation).
2. Failure to comply with special permit conditions as set forth by the Ventura County Agricultural Commissioners (CAC).<sup>3</sup> The application took place within 500 ft of occupied properties; the tarps were less than the specified thickness of 1.5 mil and the application did not end until 5:30 p.m. (in violation of a permit condition that required completion by 2:00 p.m.).
3. The grower and the AGPCO failed to comply with the supervision requirement, when operating in Ventura County (Title 3 California Code of Regulations [3 CCR] section 6406). The supervisor on duty at the time was not certified to supervise the application of restricted pesticides.
4. The pest control adviser recommended application at a rate higher than is allowed on the label for strawberry applications (a label violation).

An investigation into the incident revealed that meteorological conditions, as well as violations of the product label and county permit conditions, contributed to the exposure incident. Data from the Point Hueneme weather station (approximately 2.5 miles from the treated field) indicated that wind speed (4.0–8.1 mph) was from the east between 3:00 and 7:00 p.m. At 8:00 p.m., the wind decreased in velocity to 1.8 miles/hr, blowing from the northeast (298.5°); at 9:00 p.m. the wind speed decreased to 1.5 miles/hr, blowing from north-northeast (321.7°).

#### **4.1.3 1992 San Diego County**

On November 14, 1991, prior to planting ornamental nursery stock, an application of 67% methyl bromide and 33% chloropicrin was made at a rate of 1 lb/100 sq ft (approximately 436 lb/A) to 1 A of a 6-A field, near the north end of Encinitas. The application was made by injection, and the treated area was covered with a postapplication

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<sup>3</sup>To help mitigate exposure potential to chloropicrin, the Ventura County Agricultural Commissioner's Office had previously established the following special permit conditions for methyl bromide and/or chloropicrin field fumigation within 500 ft of occupied properties:

1. Gas-confining tarps shall have a minimum thickness of 1.5-mil or be of "high density" construction.
2. Discontinue all applications by 2:00 p.m.
3. All tarps shall remain in place for 72 hr.
4. Make no tandem applications in this area.

tarpaulin. The application rate was the maximum rate for preplant ornamental nursery applications, specified on the product label as 400 lb/A.

A woman who lived across the street, an estimated 150 ft south and east of the application site called the county sheriff's office at 7 p.m., after she began experiencing eye irritation. At 7 p.m. on November 14, 1990, weather data (from CIMIS Oceanside weather station no. 49 -33.26 latitude, -117.32 longitude, 10 miles north of incident location) showed a 1.6-miles/hr wind from north-northeast (24 degrees, with a standard deviation of 37 degrees).

In this same incident, illness also occurred in emergency responders. The sheriff's officer, who responded to the initial call, experienced eye irritation before he left the scene; he called the hazardous materials crew, according to the San Diego County Fire Department report. A fire captain, who was a member of the responding crew, was also treated that evening, for headache and eye irritation. Three additional members of his crew developed eye and throat irritation, but did not seek medical attention.

Investigation by the San Diego County Department of Agriculture resulted in a citation to the farm operator for failing to obtain a recommendation from a licensed agricultural pest-control advisor. A citation was also made for exceeding, as noted above, the maximum label rate of 400 lb/A.

#### **4.1.4 1993 Tulare County**

This episode involved an applicator, who was hospitalized for respiratory symptoms when an application rig developed a leak while his crew applied a fumigant containing 24.8% chloropicrin and 75% methyl bromide at a rate of 275 lb of product/A (68.2 lb of chloropicrin/A). He inhaled the fumes, and shortly thereafter, experienced shortness of breath. He was taken to the emergency room, treated with oxygen and hospitalized for 2.5 d for chemical bronchitis, secondary to methyl bromide/chloropicrin exposure. He also missed 7 d of work. The other driver and shovelers, who covered the tarpaulin with soil, did not experience any symptoms and only noticed an odor on their first pass through the field.

#### **4.1.5 1995 Ventura County**

In a later Ventura County episode, a grower treated a 26-A field with 100% chloropicrin, prior to planting strawberries, at a rate of 100 lb/A (2,600 total lb of chloropicrin). The application was conducted by shank injection to the strawberry beds only, and the beds were covered with a 1.5-mil thick plastic tarp. Three tractor rigs, equipped with customized chloropicrin injection equipment (chisels set approximately 8-in. deep, bed-up and tarp-laying apparatus), completed the fumigation. Irrigation lines (overhead sprinkler irrigation) were placed directly behind the tractors so that irrigation could start immediately following fumigation to get a

water seal on the furrows between the beds for 24 hr. The application started 875 ft from a housing tract and finished at 5 p.m., 215 ft from the nearest residence.

Between 7:00–7:30 p.m., residents reported watery and burning eyes, burning throat, difficulty breathing, and vomiting. A triage area was set-up and paramedics examined and treated the affected persons. Sixteen people reporting symptoms were observed, of whom five were treated on site and released. Three people were taken to the hospital and released after a few hours of observation.

In the subsequent investigation, factors contributing to the exposure incident included the late-afternoon application and the presence of a temperature inversion. (Data from the CIMIS weather station at Port Hueneme showed that the wind was 3.8–1.4 miles/hr, coming from the west, at the beginning of the incident.) No violations were found during this investigation.

#### **4.1.6 1995 Monterey County**

A small block of homes, located approximately 2.5 miles from Castroville, was affected by off-gassing from an application of 350 lb/A of 67% methyl bromide and 33% chloropicrin on July 8 and 9, 1995, to 10 A of an adjacent strawberry field. The nearest edge of the field was about 90 ft from the rear of homes on the east side of the block (Fig. 3a). Exposure was immediately noted by some residents, but was most prominent on July 10, when seams in the tarp came unglued, and a portion of the field was completely uncovered.

Eye and upper respiratory irritation were the most common problems reported by the nine residents with symptoms. Three also reported chest pain, difficulty breathing, or shortness of breath. This included one resident with sarcoidosis (a chronic restrictive pulmonary disease), who reported intermittent difficulty breathing and shortness of breath, but did not seek medical treatment. Eye irritation was also noted by an agricultural inspector and by the farm operator, who visited the application site on July 11. Prevailing winds, during the episode, were from the west, but blew intermittently from the treated field toward the adjoining residential properties (Fig. 3b).

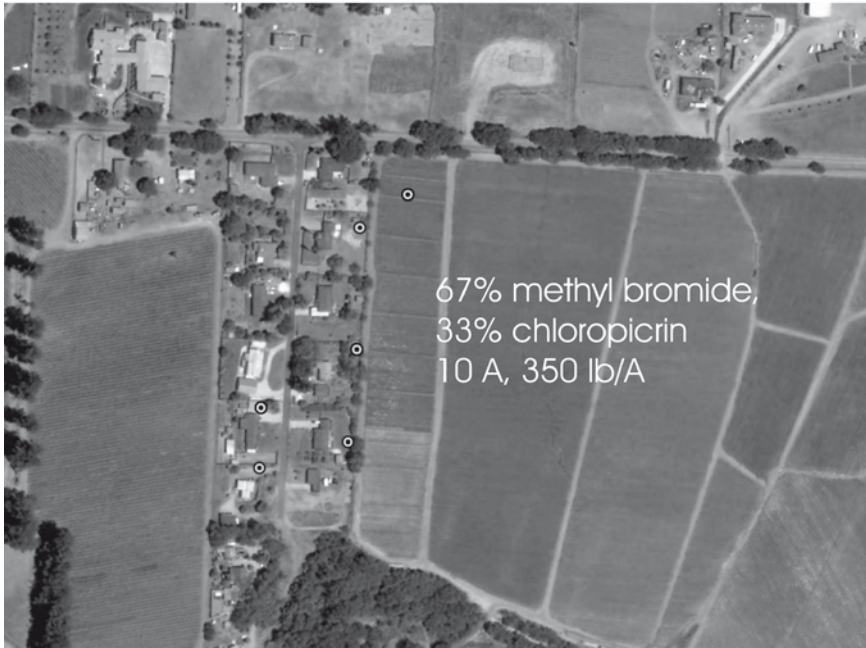
The fumigation company was fined for several violations, including failure to maintain a minimum buffer zone of 100 ft, and failure to maintain the tarpaulin used as an application site cover. The homeowners requested that future applications require a much larger buffer zone, noting a similar episode that occurred following a fumigation made in October 1993 (resulting in a complaint filed with the county agricultural commissioner's office).

#### **4.1.7 1998 Monterey County**

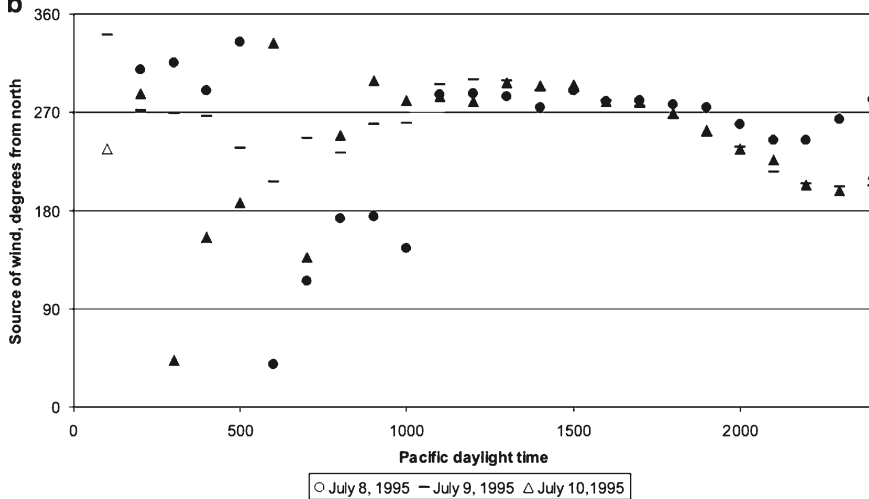
At 7 a.m. on November 1, 1997, an application of 275 lb/A was made, using 75% methyl bromide and 25% chloropicrin by shallow bed, shank injection, prior to planting 5 A of strawberries just south and west of a trailer park on the edge of Salinas. Residents phoned for emergency assistance at 12 p.m.



a



b



**Fig. 3** (a) 1995 Monterey County. Incident occurred on July 8–11, 1995, and was associated with application of chloropicrin and methyl bromide in a rural neighborhood east of Castroville, CA, in Monterey County. The prevailing wind during this period was from the west, but intermittently blew from the east during part of each day (see Fig. 3b). The “buffer”, the distance between the western edge of treated field and the nearest residential property, was approximately 90 ft. The incident shown at the northwest corner of the treated field involved a farm operator and county biologist inspecting torn sections of the tarpaulin. (b) A plot of the wind direction vs. time of day for July 8–10, 1995

Although all park residents were not systematically interviewed, there were three resident complaints of irritation from the northwest section of the park (Fig. 4a). Some residents, who lived in interior sections of the park, reported that they did not have symptoms. There were also four cases of irritation among hazardous materials specialists who responded to calls for assistance.

Weather data from an irrigation management station located approximately 2 miles southwest indicated that the wind came from the east between 10 and 11 a.m., but veered to the northeast at noon, to due north at 1 p.m., then to the northwest by 2 p.m. By 7 p.m., the wind shifted to again blow from the east (Fig. 4b).

The county department of agriculture fined the applicator because the measured buffered zone, between the edge of the application and the trailer park, was 17 ft; the minimum required by permit conditions was 30 ft.

#### **4.1.8 1999 San Joaquin County**

On April 15, 1999, an application of 56.8% methyl bromide and 42.2% chloropicrin (350 lb/A) was made by shallow shank injection (with a postapplication tarpaulin) prior to planting 6.4 A of nursery strawberries, in a sparsely populated area south of French Camp.

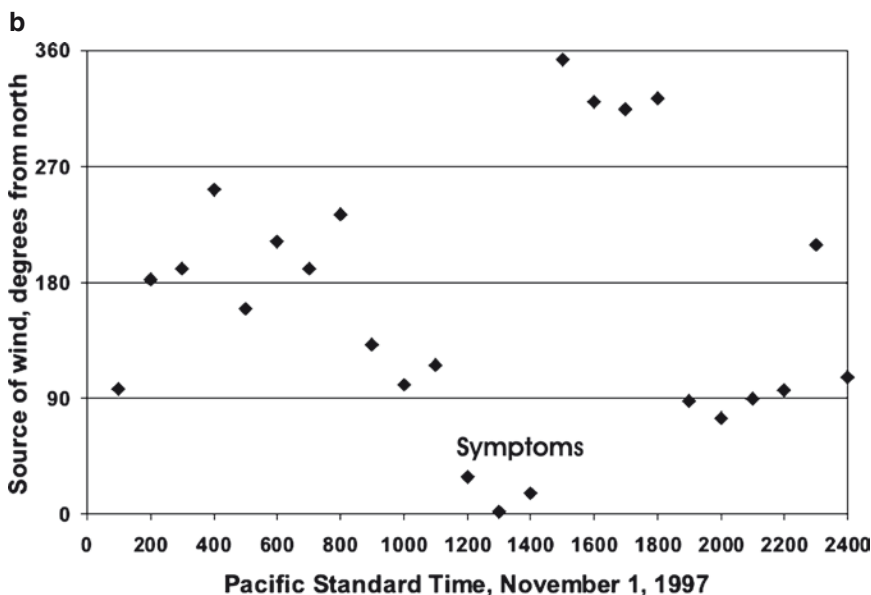
Beginning at 8:30–9:00 p.m., irritant eye and upper respiratory symptoms occurred in three family members, who lived just west of the treated field, and in an employee of a truck stop located farther west along a freeway service road. That evening, two emergency workers suffered similar eye and upper respiratory symptoms after responding to the call.

Data from the Lodi station, 17 miles north, and slightly west, showed 1–2.5 miles/hr wind from the southeast between 7 and 11 p.m., with a variation of 23–41 degrees. The Manteca station, 3.4 miles southeast, showed a 1.1–2-miles/hr wind from the south, but with a variation of 23–72 degrees. The National Weather Service archive for the Stockton Airport station (located about two miles northwest of the fumigation site) recorded the wind as variable at 7 p.m., blowing from the east between 8 and 9 p.m., and then blowing southwest between 10 and 11 p.m. Wind speeds varied between 3 and 5 miles/hr.

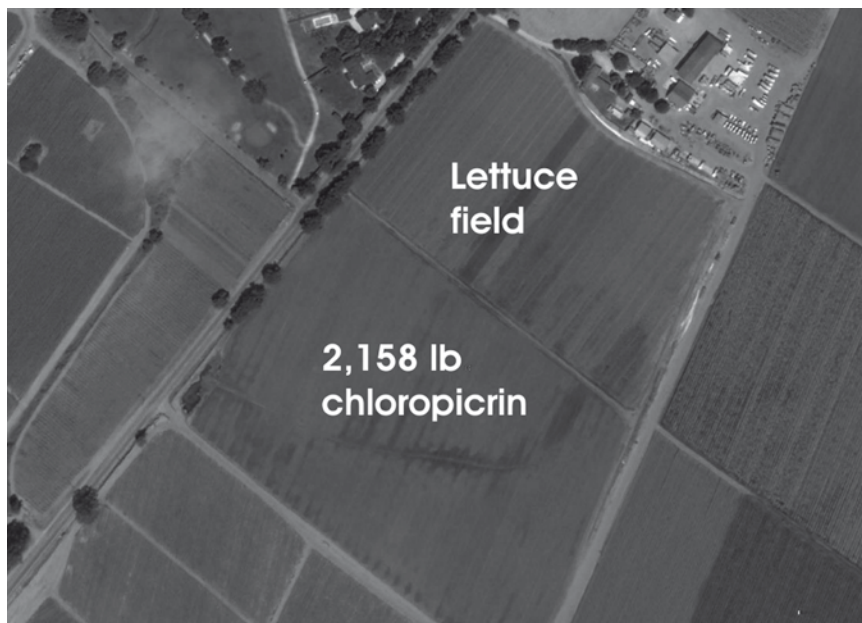
Investigation did not reveal any violations that contributed directly to resident illnesses among those who lived to the west of the treated field. Stable atmospheric conditions were probably related to the incident. However, a citation was issued for violation of the 200-ft buffer zone, based upon a measurement confirming that the nearest house to the east was 137 ft from the treated area.

#### **4.1.9 2000 Monterey County**

In Monterey County, in 2000, four crews, consisting of approximately 152 workers were harvesting lettuce; these workers were moving toward a neighboring 16-A field (Fig. 5) that was under fumigation since the previous day. The fumigation was



**Fig. 4** (a) 1998 Monterey County. Five A were treated south and west of a trailer park located on the edge of Salinas, CA. Treatment was made between 7 a.m. and 12 p.m. on November 1, 1998, with 75% methyl bromide and 25% chloropicrin at a rate of 275 lb/A. The distance between the treated areas of the field and the trailer park listed in the permit condition was 30 ft, but the measured distance was as close as 17 ft. Wind during the episode was variable, but blew from the north and east at the outset of the episode (Fig. 4b). (b) Data from CIMIS weather station 116, located approximately 2 miles southwest of the treated site; wind speed was 9 miles/hr at 12 p.m., but slowed to less than 5 miles/hr later in the afternoon



**Fig. 5** 2000 Monterey County. Nine field workers of a crew of approximately 150 became ill while harvesting lettuce downwind from a 16-A field first treated with 49.5% methyl bromide and 41.5% chloropicrin, at a rate of 325 lb/A, then covered with a tarp. The wind was from the southeast at 8 miles/hr at the onset of the incident

being performed with a mixture of 49.5% methyl bromide and 41.5% chloropicrin, at a rate of 325 lb/A, and the treated site was covered with a tarp. The permit conditions for the fumigated field required notification to owners of the adjoining property, but no such notice was made to the lettuce grower.

Of nine fieldworkers in this event who reported symptoms that included watery and burning eyes, sore throat, difficulty breathing, nausea and vomiting, six were taken to a nearby hospital by ambulance. One worker, with pre-existing asthma, had difficulty breathing, then fainted. She had some wheezing in the emergency room and was hospitalized for overnight observation. Another worker was 3 months pregnant. Some emergency responders noted transient eye irritation while they were at the site. Air measurements for methyl bromide taken at the site were negative.

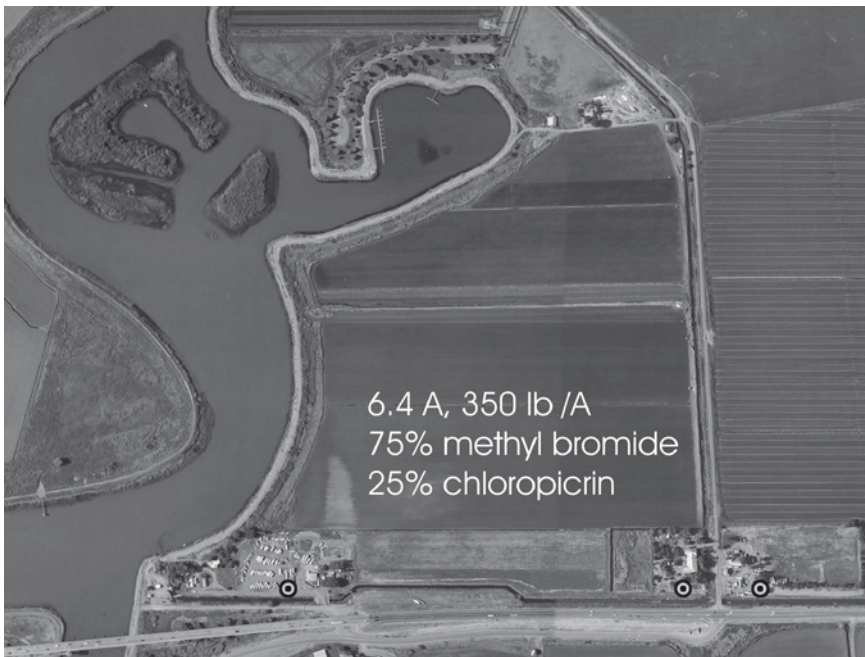
Data obtained from a local weather station showed that, in the early morning, it was calm and cloudy. At 8:00 a.m., when the crew first reported symptoms, wind speed had picked up and was coming out of the southeast at 8.2 miles/hr from the treated field toward the direction of the lettuce field. Also noted was that the temperature rose over 10 degrees between 8:00–10:00 a.m., but the ground temperature remained cool, typical conditions for a temperature inversion to occur. The road separating the treated and lettuce fields was only 12 ft wide, and it appeared that

some lettuce had been cut by the workers near the south edge of field. The symptomatic farm workers were working on two lettuce mobile harvesting and packing stations, “lettuce machines”, one located 160 ft and the other 250 ft from the edge of the treated field.

The CAC investigation noted that the application company violated the restricted materials permit conditions for methyl bromide, specifically a requirement to notify owners of adjacent agricultural properties that fall within a specified worker buffer zone. The size of the buffer zone depends on the number of acres to be fumigated, the amount of methyl bromide to be applied per acre, the slope of the field, and the presence of any other fumigations in the area. The buffer zone in this episode was 30 ft from the edge of the treated field and was in effect for 24 hr after the application.

#### 4.1.10 2001 San Joaquin County (22-Sj-01, Index Case 2001–307)

A drift episode, in 2001, involved 10 people living near the Mokelumne River, west of Lodi. Individuals involved lived just south of a 6.4-A tract that was undergoing shank and tarp fumigation (unknown tarp thickness) on April 14, 2001, with 350 lb/A of 25% chloropicrin and 75% methyl bromide (Fig. 6).



**Fig. 6** 2001 San Joaquin County. Site of an April 14, 2001, application resulting in an incident following shank and tarp application of chloropicrin and methyl bromide



The application permit required a worker buffer zone of 100 ft, and a residential buffer zone of 480 ft. Residents reported a north wind at the application site. Data from a weather station 5.8 miles west showed that the wind blew 1–4 miles/hr from the west and northwest most of the day, with hourly variations of 11–60 degrees.

Members of the exposed group experienced symptoms throughout April 14, including intense eye and upper respiratory irritation, headache, nausea, chest pain, chest congestion, and asthma. Members of one family evacuated their home in the middle of the night. Several reported symptoms for several days thereafter.

An investigation into the incident revealed several possible contributing factors:

1. Both residents and CAC staff observed several tears in the tarp applied after the application.
2. Wind blowing in the direction of the residential area aided in carrying the pesticide off-site, and low wind speeds caused vapors, escaping from the treated field, to remain in the immediate vicinity of the application.
3. The grower fumigated an area larger than allowed in the worksite plan. The actual distance from the edge of the treated area to houses located to the south measured 185 ft, well inside the required residential buffer zone.

#### **4.1.11 2001 San Luis Obispo County**

In San Luis Obispo County, in September 2001, an AGPCO treated 4 blocks (a total of 30.5 A) of a preplant strawberry field by shank injection with a fumigant containing 42.6% chloropicrin and 57% methyl bromide at a rate of 250 lb of product/A (106.5 lb of chloropicrin/A) over a 7-d period. After the fumigation, the treated area was covered with 1.25-mil tarps. The initial application began at 7:50 a.m. on September 30, 2001, and was concluded by 9:15 a.m. The field was surrounded on four sides by residential development (Fig. 7).

A man, residing 200 ft south of the application site, called to report an odor at 8:57 a.m. on September 30, 2001. He stated that the odor began before 8 a.m., and continued until 9 a.m., when a shift in the wind began pushing the odor to the east. The man and his wife were affected by the odor. An additional complaint was received from a resident approximately 400 ft south of the eastern edge of the field, and one from a resident 800 ft to the east. Symptoms reported from the drift incident included shortness of breath, dry cough, asthma, chest pressure, runny nose, headache, and lack of energy.

Seven additional complaints, representing possible postapplication off-site movement, were received regarding symptoms experienced on September 30, and October 2 and 8. The distribution of the complaints, principally occurring south and east of the treated field, was very similar to that of the drift cases. One postapplication complaint derived from a residence 140 ft west of the treated field. Symptoms experienced were similar to those reported in the drift cases, including aggravation of asthma, shortness of breath, persistent cough, and headaches. The pattern of symptoms was atypical in that no cases of eye irritation were reported.



**Fig. 7** San Luis Obispo County. Distribution of incidents associated with drift and off-site movement, reported following application of methyl bromide and chloropicrin, in September of 2001 and 2002. Squares: 2001 drift associated cases (occurring during an application); triangles: 2001 cases associated with postapplication off-site movement; crosses: 2002 drift associated cases; circles: 2002 cases associated with post-application off-site movement

Staff from the local CAC had monitored the applications on September 30, and October 2, 4, and 6 by visual observation and also by noting the presence or absence of odor (this was apparently done because of complaints regarding previous pesticide applications made at the site). CAC staff did not identify any violation of the application permits, also reporting that they did not detect any odor personally, or experience any symptoms.

Investigation of the episode was limited by the absence of a nearby weather station (the closest station was approximately 15–18 miles distant, separated by a coastal mountain range to the north). The staff had no means of collecting air samples for either methyl bromide or chloropicrin. No survey was conducted to search out unreported cases in the neighborhoods surrounding the field.

#### 4.1.12 2002 San Luis Obispo Country

In September 2002, 28 A of the field that was the source of the 2001 (described immediately above) complaints were fumigated in four sets over a 12-d period

(from 5 a.m. to 10 p.m. on September 20, 23, and 28, 2002, and on October 1, 2002). The treatment material contained 19.8% chloropicrin and 80% methyl bromide, and was applied at a total rate of 240 lb of product/A- equivalent to 47.5 lb of chloropicrin/A. The CAC monitored each application and identified no violations. During the 2002 fumigation, the application company operated a weather station at the site, recording wind speed, wind velocity, and temperature. The wind speeds recorded were usually less than 5 miles/hr and often not measurable; wind direction was highly variable.

A total of 14 residents complained of symptoms throughout the application period. As with the 2001 episode, systemic complaints were common and there were no cases of eye irritation reported. The case distribution also closely resembled the one seen in the 2001 episode (Fig. 7).

The initial case was reported on September 20, 2002, after treatment of the initial block of 7 A at the south end of the field had concluded. There were eight additional postapplication cases and five drift cases. The drift and postapplication cases occurred in similar distribution, principally south of the treated field. One case occurred along the road just north of the treated field, and was reported by a driver passing through the area on the evening of September 27, 2002. The absence of other reported cases from the north area is noteworthy, considering that low wind speeds and variable wind direction existed throughout the application period. No survey for unreported cases was conducted either north or south of the treated field.

#### **4.1.13 2003 San Joaquin County**

In San Joaquin County on February 11, 2003, an AGPCO treated 20.7 A on French Camp Road, with a fumigant consisting of 34.7% chloropicrin and 61.1% 1,3-dichloropropene. The application was performed at a rate of 115 lb of product/A (about 40 lb of chloropicrin/A), using a nontarped, shallow injection (12-in. deep). Two houses were located west of the field just outside the 100-ft buffer zone, as required by the application permit (the closest house was located approximately 100 ft from the treated field, and the other house was 160 ft distant).

At 5 p.m., the occupant of one house contacted the Sheriff's office to complain of an odor, and then was directed to call the fire department and the local Air Pollution Control District. Records from the CIMIS weather station, located approximately 6 miles to the northwest, indicated that the wind was from the east (at 5 p.m.). A total of 12 residents suffered eye irritation and sore throats that subsided within 2 d of the incident. None required medical attention.

Between 6 and 8 p.m., the wind began coming from the northwest. The fire crew arrived at 6:30 p.m. and was unable to detect the odor reported by the residents. An investigation conducted by the local CAC did not reveal any violations of the fumigant label instructions or permit conditions, but the grower's permit was subsequently modified to include written notice to nearby residents, and at least a 300-ft buffer zone.



#### 4.1.14 2003 Kern County

In Kern County on October 3 and 4, an AGPCO applied 100% chloropicrin at 80 lb/A to 34 A of fallow land (prior to planting onions). Chloropicrin was injected into the soil 17–18 in. deep. A tarp was not required for this type of application; a weighted board was pulled behind the shanks in an attempt to compact the soil. The site was approximately ¼ mile north of a housing complex, an unoccupied migrant labor camp, scattered single-family homes, and an elementary school. Single-family homes and a small apartment complex also lay approximately ¼ mile to the west of the treated field (Fig. 8a).

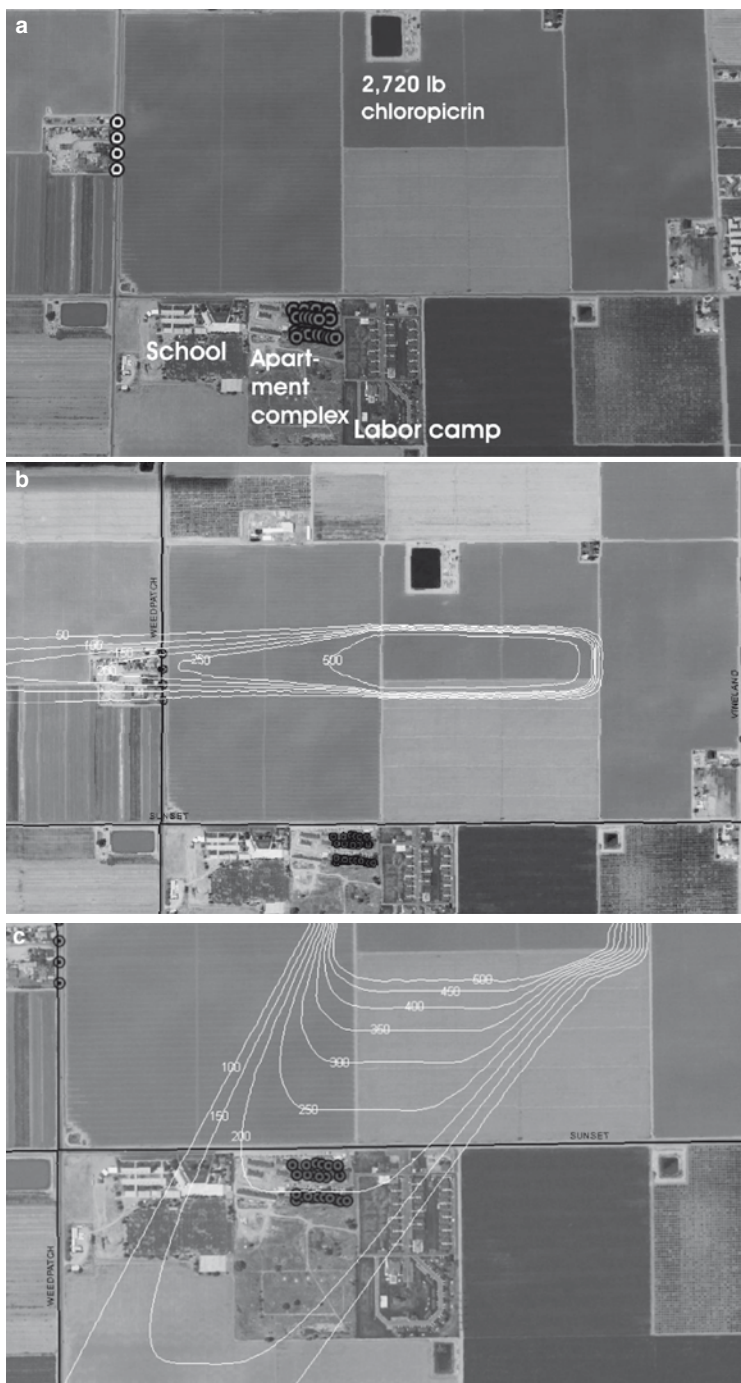
On October 3, the application was stopped after fumigating 18 A at approximately 6:00 p.m. as a result of impending darkness. Records from a weather station approximately three miles to the east of the treated site indicated that the wind direction on October 3 varied between northeast, east, and southeast, and wind speeds were less than 5 miles/hr. Residents from the apartment complex and single-family homes to the west began experiencing irritation, systemic and respiratory symptoms; these prompted a 911 call and response by the county fire department hazardous materials team. Upon arrival at the location of the residents, the emergency responders did not experience any irritant symptoms. Because of the darkness and the ¼-mile distance from the treated field, they were unable to determine the source of complaints. Just before midnight, the wind veered to blow from the northwest and the problem abated.

The application resumed the following day at approximately noon and was completed at about 6:00 p.m., leaving a 60-ft buffer around the field edge. Complaints were again received from the residents living west of the treated field that evening as the wind shifted directions, blowing from the treated field toward the residential area. Residents of an apartment complex located ¼ mile south of the treated field, and adjacent single-family homes, also reported symptoms. The county hazardous materials emergency team again responded. They evacuated residents of the apartment complex to a staging area about one mile away. Estimated air concentrations in the vicinity of the apartments ranged between 150 and 200 ppb of chloropicrin for the period between 7 and 8 p.m. on the evening of October 4.

From October 3 through October 4, 153 residents (of the 172 residents in the immediate area) reported chloropicrin-compatible symptoms: 151 (99%) had irritant symptoms (eye and upper respiratory tract); 85 (56%) had nonspecific systemic symptoms (nausea, vomiting, headache, etc.); 66 (43%) had symptoms potentially related to the lower respiratory tract (cough, difficulty breathing, or chest pain); five had a prior history of asthma, and experienced mild to moderate exacerbations of symptoms, during the event. When the residents were initially interviewed (October 6–7), only 11 (7%)

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**Fig. 8** (a) Site of the October 3–4, 2003, incident associated with use of 100% chloropicrin on 34 A at an application rate of 80 lb/A. The farm labor camp (bottom center) was vacated on September 30, 2003, just before the incident. The elementary school was also vacant because the incident occurred on Friday and Saturday evenings. (b) An aerial photograph of an air pollution plume taken at 7–8 p.m. on the evening of October 3, 2003, near the intersection of Sunset Blvd and Weedpatch Highway, south of Lamont, CA. Contour lines represent varying concentrations of chloropicrin in parts-per-billion (ppb). (c) Plot of chloropicrin concentration isopleths (ppb of chloropicrin)



received some type of medical evaluation. These included two individuals who were evacuated and sent for medical care by paramedics on the evening of October 4. Several of those exposed, including two children, less than 1 yr of age, had persistent respiratory symptoms at the time a community meeting was held on October 15. However, because most had no medical insurance, follow-up medical care was limited (O'Malley et al. 2004b). Eight emergency responders, the farm owner, the lead investigator, and three application crew members all reported eye irritation, following exposure during this incident. One of the applicators also experienced difficulty breathing.

No exposure measurements were taken at the time of the incident, but Department of Pesticide Regulation made estimates retrospectively using a standard air dispersion model (ISC3) (Barry and Walters 2003), and flux estimates made during the 1996 registrants study. Estimated air concentrations on October 3, for the period between 7 and 8 p.m., along Weedpatch Highway, west of the treated field, ranged from 200–250 ppb of chloropicrin (Fig. 8b). For the evening of October 4, estimated chloropicrin concentrations ranged from 150–200 ppb along Sunset Boulevard (Fig. 8c). Estimates of peak exposure levels, based on peak-to-mean extrapolations described by Hino (1968), indicated that peak concentrations exceeded 1 ppm on both evenings. No comparable estimates were available from any of the previous episodes.

The most significant identified violation of pesticide regulations was failure to adequately compact the soil, following applications to seal in the fumigant. A temporary local prohibition on the use of chloropicrin was imposed by the Kern County Department of Agriculture following the incident (O'Malley et al. 2004b), which was lifted following development of new permit conditions.

#### **4.2 Off-site Movement of Chloropicrin, Without Associated Complaints of Drift**

On September 9, 2003, an AGPCO applied 5,640 lb of chloropicrin (94% EC formulation) through a drip system to a 30-A field (to be planted with strawberries), adjacent to the maintenance yard of an Orange County school district. The next morning, 14 school district employees suffered irritated eyes upon arriving at the transportation yard adjacent to the field. During the morning of the incident the wind was blowing from east to west; a triangular portion of the south end of the field was directly upwind of the northwest portion of the maintenance yard (Fig. 9).

No evidence of permit condition violations was noted in investigation of this incident. No buffer zones are required for applications of 94% chloropicrin such as those that are used when mixtures of methyl bromide and chloropicrin are applied.

### **5 Chloropicrin Incidents Outside of California**

Chloropicrin exposure incidents, similar to those reported in California, have been reported for other locations in the literature, and these have documented effects of drift into nontarget areas.



**Fig. 9** Priority episode 31-ORA-03: The photograph shows a fumigated field that is located adjacent to a school district maintenance facility. This incident took place near the intersection of Interstate 5 and Highway 133, following application of 94% chloropicrin to an adjacent field on the previous day. The lower portion of the treated field (below the line) was directly east of the northeast corner of the yard, where employees experienced illness on the morning of September 10, 2003. Data from CIMIS weather station 75, located 1.6 miles east of the fumigated field, reported wind was from the east throughout the morning (90° = wind from East, 180° = wind from South, 270° = wind from West)

In Japan, residents living near and over 100 m (328 ft) from a site, where leaf tobacco was fumigated with chloropicrin, complained of excessive lacrimation, vertigo, cough, headache, nausea, vomiting, and fatigue at the time of their exposures. Some symptoms lasted for several hours, and some persisted for up to 3 d postexposure. A few people reported that their symptoms lasted for more than 1 month (Okawada et al. 1980).

In 1989, the case of a Belgian farmer, who received a container of chloropicrin mislabeled as metam-sodium from his pesticide supplier, was reported. He consequently unintentionally mixed this product with a previously purchased batch of metam-sodium and used it to fumigate a greenhouse. The vapors escaped through the vents of the greenhouse and, with the aid of a low-velocity wind, dissipated into neighboring areas. A large number of animals (2,500 turkeys, numerous ducklings, four sheep, and a goat) adjacent to the greenhouse died from exposure to the gases.

No human fatalities were reported, but residents within 200–600 m (656–1967 ft) of the greenhouse reported various complaints, including eye irritation, lacrimation, coughing, runny nose, nausea, sore throat, headache, dyspnea, and skin irritations. Thirty-five people, primarily rescue workers, were admitted at a hospital emergency unit and examined. Based upon the complaints and previous estimates of dose–response from the medical literature, the investigators estimated that the air concentration of chloropicrin was between 0.05 and 0.1 mg L<sup>-1</sup> (7.5 and 15 ppm, approximately) (Selala and Janssens 1989).

## 6 Management of Irritation from Chloropicrin Exposure

The irritation caused by chloropicrin at concentrations below 100 ppb (Cain 2004) presents a difficult management problem, highlighted by the priority episodes previously described, where no application violations were noted (i.e., the following years and California counties: 1992 Merced, 1995 Ventura, 2001 San Joaquin, 2001 San Luis Obispo, 2003 San Joaquin). Although the concentrations that provoked symptoms were not determined in most of these episodes, off-site monitoring studies suggest downwind air concentrations that exceed the demonstrated irritation threshold may be common.

Among the reported cases, the most distant downwind measurements in the monitoring studies were less than 200 ft from the treated fields. To determine air concentrations further downwind would require additional air sampling, or estimates of the concentrations using air modeling techniques. The importance of gaining such additional insights through an appropriate mechanism is underscored by the seriousness of the complaints (painful eye and respiratory irritation) that have been reported  $\frac{1}{4}$  mile away from the application site in the Kern County incident of 2003.

Possible mitigation measures are currently being evaluated in the California Department of Pesticide Regulation risk assessment process. Such mitigation measures may include limitation of applications during adverse weather conditions (especially low wind speed or temperature inversions). As described in several cases following tarped fumigation, climatic conditions were initially still, foggy or overcast prior to the arrival of a light wind, which usually led to much higher probability of having symptoms reported. Stable atmospheric conditions, with the characteristic of little or no vertical air mixing, cause the off-gassing fumigant to remain in a concentrated plume for a considerable distances from the application site. As illustrated by the San Luis Obispo County episodes in 2001 and 2002, and the Orange County 2003 episode, postapplication off-site movement may also be an important source of exposure for businesses and residential areas downwind of treated fields.

Other restrictions written into county level permit conditions include completing applications by early afternoon (to minimize the effects of temperature inversions), prohibiting tandem tractor applications, and limiting the number of treated acres.

Buffer zones can also be used to protect people from drift and off-site movement of fumigants. These must take into account evolving patterns of land use: as a result of the rapid conversion of farmland into residential space, homes and apartments may be located in close proximity to agricultural fields (O'Malley et al. 2005). Buffer zones, to protect workers in adjacent agricultural fields, may also be necessary.

Newer application techniques also offer some potential for decreasing fumigant emissions. The most promising experimental results have been associated with drip applications made 30 cm below the soil surface. In field experiments with metam-sodium (Papiernik et al. 2004), this application method reduced peak methyl



isothiocyanate flux from 1.5  $\mu\text{g}/\text{m}^2/\text{sec}$  (for a 15-cm depth subsurface drip application) to 0.08  $\mu\text{g}/\text{m}^2/\text{sec}$ . Whether broad-scale commercial applications of this technique are feasible remains to be established. Current drip application techniques have been associated with both drift<sup>4</sup> (post-application offsite movement) and off-site movement (2003 Orange County).

## 7 Summary

With limitations imposed on the use of methyl bromide by international treaty, use of metam-sodium, chloropicrin, and other fumigants have increased; this increase has been accompanied by multiple community illness episodes. In this review we address the California experience of direct or indirect exposures to chloropicrin, after use of this fumigant as an active ingredient in agricultural pest control, from the years 1992–2003.

The best available toxicology data demonstrate that, for brief exposures (5–30 sec), eye irritation (or at least “chemesthesis” – detection of exposure by the eye) occurred in 50% of volunteer subjects exposed to 700 ppb of chloropicrin. Of 62 subjects tested, 10–15% (depending upon the site of exposure) failed to detect 1200 ppb of chloropicrin (the highest concentrations tested). For exposures lasting 20 min, the minimum concentration detectable by 50% of the subjects was 75 ppb; the no-observed-effect-level (NOEL) for eye irritation was 50 ppb. Exposures for 1 hr, at 100 and 150 ppb, produced subjective eye irritation in most subjects. Using a standard tenfold uncertainty factor (Exttoxnet 2006), an estimated NOEL for a 1-hr exposure to chloropicrin (calculated from the 100 ppb lowest-observed-effect-level) would be 10 ppb.

The few monitoring studies conducted for agricultural use of chloropicrin demonstrate that the most sensitive effect to chloropicrin exposure is sensory irritation.

The use of chloropicrin for field fumigation presents a difficult problem, both because of the extreme degree to which it is irritating, and because of the very low concentrations that cause such irritation. The cases summarized in this report, especially those resulting from applications that adhere to label and permit standards, suggest that additional mitigation measures are needed to minimize off-site human exposure associated with chloropicrin applications in California. Examination of the previously discussed Pesticide Illness Surveillance Program cases revealed that conducting the applications according to label directions and local permit conditions was not always adequate to prevent off-site exposure or resultant irritation or other symptoms. This suggests that current methods of fumigant containment and/or buffer zone requirements are insufficient under some circumstances.

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<sup>4</sup>An episode affecting 320 residents was associated with a drip application on the north end of Salinas, CA, in September, 2005. A detailed description of the episode is under review.

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# Adverse Health Effects of Pesticides in Agrarian Populations of Developing Countries

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## 1 Introduction

In low- and middle-income countries, and across the world, the need to ensure local agricultural production and food security, while simultaneously protecting the population against health affects from pesticide exposure, has emerged as a major public health challenge. As agricultural production in Africa intensifies, and as pesticide use becomes more widespread, an increase of pesticide poisoning cases is to be expected (London et al. 2005). Acute poisoning by agricultural pesticides is currently an important cause of human morbidity and mortality worldwide, with some 25 million farm workers annually exposed to pesticides in developing countries (Jeyaratnam 1990).

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Developing countries use only 20% of the world's agrochemicals, yet they suffer 99% of deaths from pesticide poisoning (Jeyaratnam and Chia 1994). The World Health Organization (WHO) estimates that at the global level, 3 million severe pesticide poisoning episodes occur annually, and of these, a minimum of 300,000 people die, with 99% of cases being from low- and middle-income countries (Gunnell and Eddleson 2003). Japanese farmers use an estimated 400,000 t of pesticides per annum, and the number of deaths caused by such pesticide use is about 1,000 persons per annum (in the early 2000s; Nagami et al. 2005). The Poison Information Centre of the National Institute of Occupational Health, in Ahmedabad, reported that organophosphorus (OP) pesticides were responsible for the maximum number of poisonings (73%) among all agricultural chemicals (Dewan and Sayed 1998).

India is the most prominent manufacturer of basic pesticides in Asia, and ranks 12th globally in pesticide production. Insecticides account for 75% of India's total pesticide consumption, followed by fungicides (12%) and herbicides (10%). More than 50% of the total quantity of pesticides used in India is sprayed on cotton, 17% on rice, and 13% on vegetables and fruits (Indra et al. 2007). The active ingredients of 44% of the 265 different pesticides used in Indonesia are categorized by the WHO as being extremely, highly or moderately hazardous (Table 1) (Kishi et al. 1995).

**Table 1** Pesticides commonly used in developing countries and their categorization by WHO (World Health Organization) hazard class

Pesticide: Common name (WHO toxicity classification)	Chemical family
Ia Extremely hazardous <sup>a</sup>	
1. Phorate	Organophosphate (OP)
Ib Highly hazardous <sup>a</sup>	
2. Monocrotophos	OP
3. Profenofos and Cypermethrin	Combination of an OP and a synthetic pyrethroid
4. Carbofuran	Carbamate
II Moderately hazardous	
5. Dimethoate	OP
6. Quinalphos	OP
7. Endosulfan	Organochlorine (OC)
8. Carbaryl	Carbamate
9. Chlorpyrifos	OP
10. Cyhalothrin	Pyrethroid
11. Fenthion	OP
12. DDT	OC
III Slightly hazardous	
13. Malathion	OP
IV Unlikely to present acute hazard in normal use	
14. Carbendazim	Carbamate
15. Atrazine	Triazine

<sup>a</sup>WHO Classification of pesticides (2004)

## **2 Concerns About Pesticides in the Agrarian Environment**

The pesticides used in agricultural areas reach the environment and humans directly or indirectly. Humans are exposed to pesticides via environmental media (contact with soil, water, air, and food) and can enter the body by three different routes: inhalation, ingestion, and dermal contact. Acute and chronic poisoning, among those applying pesticides, continue to be prominent health hazards in rural areas of developing countries. However, published reports that address these hazards in these countries are rare. The effects of poisoning may range from temporary acute effects such as eye irritation and excessive salivation, to chronic diseases such as cancer, or reproductive and developmental disorders (Yassi et al. 2001). Some pesticides have been implicated as endocrine disruptors and are known to elicit their adverse effect by mimicking or antagonizing natural hormones in the body. It has been postulated that long-term, low-dose exposure to such agents may be linked to human health effects such as immunosuppression, hormone disruption, diminished intelligence, reproductive abnormalities, and cancer (Crisp et al. 1998).

Acute pesticide poisoning is not as pronounced in Africa as it is in Asia. Currently, there are no precise estimates of the full impact to health from longer-term pesticide exposure. The practices of those who spray pesticides in developing countries are often inadequate and result in substantial exposure to the farmers who use these agents; moreover, signs and symptoms occur significantly more often during the spray season than during periods when no applications are being made (Kishi et al. 1995). Neurobehavioral and respiratory symptoms, and intestinal problems in these farmers were significantly and independently associated with the following factors: number of spray operations made per week, the use of more hazardous pesticides, and the wetting of skin and clothes with spray solutions (Kishi et al. 1995). Although the pesticide used varies with season and crop produced, there is continuous usage of different pesticides throughout the year in developing countries; among the pesticides used are those banned elsewhere. Household pesticides are often used without prior knowledge of their toxic effects, and without use of safety precautions, which can lead to adverse health problems (Jamil et al. 2007).

## **3 Pesticide Poisoning Incidents and Farm Practices in Developing Counties**

More than half of the population (56.7%) of India is directly engaged in agriculture, and is directly exposed to agricultural pesticides (Gupta 2004; Government of India 2001). India ranks second among Asian countries in annual pesticide consumption (O'Malley 1997). As early as 1958, one author reported that more than 100 people died in Kerala after consuming wheat flour contaminated with ethyl parathion (Folidol E 605) (Karunakaran 1958). In another outbreak, in 1977, eight cases of grand mal seizures were reported from an Uttar Pradesh village following accidental

ingestion of hexachlorocyclohexane (HCH)-contaminated wheat (Nag et al. 1977). Of the 35 human cases of malathion poisoning reported during 1967–1968, five individuals died. In Indore, ECG (electrocardiogram) changes were recorded in all pesticide poisoning cases, and autopsy and histopathological studies revealed damage to the myocardium of exposed individuals (Sethuraman 1977).

OP insecticides are characterized as being easily absorbed by respiratory and gastrointestinal mucosa, and OP compounds are readily absorbed through the skin (Karalliedde 1999). OP insecticides commonly used in agriculture are malathion, parathion, dimethoate, chlorpyrifos, monocrotophos, and quinalphos. The mortality rate observed following poisoning by OP insecticides varies between 4 and 30% (Yamashita et al. 1997).

OP compounds are commonly used as insecticides and fungicides in Kashmir, India (Malik et al. 1998). Poisoning incidents have been observed for pesticides such as phosphamidon (55% cases), malathion (12.2% cases), dichlorvos (8.5% cases), Tic-20 (10.4% cases), and an unknown agent (13.4% cases) (Malik et al. 1998). Pesticide exposure may result from ingestion (proportionately, 85.4%), inhalation (proportionately, 4.3%), and topical (dermal) contact (proportionately, 10.4%). Self-poisoning, with suicidal intent, is a major problem in Sri Lanka and India, and is responsible for over 90% of reported poisoning incidents (Senanayake and Karalliedde 1986; Malik et al. 1998). Such incidents occur mainly among males (Senanayake 1998) as a result of financial crisis or crop loss (Jeyaratnam 1990; Eddleston 2000; Eddleston and Phillips 2004).

The increasing and indiscriminate use of OPs as agricultural and household insecticides, without accompanying public education on storage and safe use, increases the potential for poisoning incidents (Chaudhary et al. 1998). In India, 51% of food commodities are contaminated with pesticide residues and of these 20% have residues that exceed the legal maximum residue limit (Gupta 2004). The long-term, low-dose exposures to some pesticides are increasingly linked to human health effects such as immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities, and cancer (Gupta 2004). The study conducted by Jamil et al. (2007) suggests that Indians living in rural areas are more highly exposed to the hazardous effects of pesticides. Table 2 presents an overview of incidental and occupational (agriculture) pesticide-poisoning cases, in developing countries, along with the consequence of these exposures.

Pesticide poisoning is a significant problem in developing countries primarily because of inadequate, inconsistent, or poor work practices that are normally necessary and appropriate to assure safety. In addition to exposure during pesticide applications, workers are also exposed in various chemical handling operations: mixing, cleaning, and loading spray equipment, and when disposing of empty containers. Less than 2% of pesticide applicators understand how toxic the pesticide formulations they spray actually are (Indra et al. 2007; Ejigu and Mekonnen 2005). Only about one third of applicators read the instructions for pesticide use marked on the containers; even fewer (2.5%) take steps to follow those instructions. A mere 1.5% of applicators understand the color code system, used in the region, to inform them of pesticide toxicity level. Moreover, the applicators who are aware of the potential health hazards

**Table 2** A survey of cases and consequences of incidental and occupational (agriculture) exposure to pesticides in developing countries

No.	Type of pesticides	Effects and symptoms	Country; reference	Exposure time (yr)	Type of poisoning - incidental or occupational
1	DDT, DDE and traces of lindane, Arachlor, heptachlor, aldrin, endrin, hexachlorobenzene (HCB)	Precocious puberty (endocrine disorder) among exposed children	Belgium; Konstantinova et al. 2001	9	Incidental
2	Methamidophos, carbofuran, monochrotophos	Acute poisoning led to mortality of 12.98%	Brazil; Maria et al. 2006	10	Occupational (agricultural)
3	2-4-D, picloram, glyphosate, benomyl, chlorothalonil, paraquat, carbofuran, proconazole, mancozeb, terbufos, methamidophos, deltamethrin, methomyl, triadimefon, fluazifop, captafol, lead arsenate, fenamiphos, phoxim, malathion, dichlorvos, terbutylazine, diuron, oxamyl, quitozene, aldrin	Childhood leukemia	Costa Rica; Patricia et al. 2005	21	Occupational (agriculture)
4	Chlordimeform, chlorpyrifos, diazinon, fenthion, fenthion, sulfoxide, mephosfolan, methyl parathion, p,p'-DDE, p,p'-DDD, o,p'-DDT, p,p'-DDT, $\beta$ -HCH, lindane ( $\gamma$ -HCH), toxaphene	Decreased cholinesterase activity among exposed children	Nicaragua; Connell et al. 1999		Incidental
5	OP and carbamate pesticides	Headache, burning sensation in eyes/face, weakness, fever watering eyes, blurred vision, skin, irritation/itching, dizziness, nausea and vomiting, cold/breathlessness/chest pain, forgetfulness, male impotence, female infertility, decreased cholinesterase activity	Ghana; Clarke et al. 1997	1-21	Occupational (agricultural)
6	Methyl bromide, methamidophos (Tameron®), DDVP (dichlorvos), folimat, captan, folidol, bazudin, agrimec, others (pyrethroid, carbamate, chlorohydrocarbons)	Fatigue, headache, changes in mood, irritation in the eye, blurring of vision, difficulty breathing, pressure on the chest, coughing, mucous saliva, skin itch, scars, dizziness, depression, forgetfulness, memory disorders, sleeplessness, nausea, vomiting, bleeding nose, weight loss, deformity of nails	Turkey; Ergonen et al. 2005	14	Occupational (agricultural)

(continued)

Table 2 (continued)

No.	Type of pesticides	Effects and symptoms	Country; reference	Exposure time (yr)	Type of poisoning - incidental or occupational
7	Cartap, isoprothiolane, fenobucarb, thiosultap-sodium, difenconazole, sulfur, isoprothiolane, pretilachlor, butachlor, fthalide (phthalide), iprodione, tricyclazole, carbendazim, acephate, diazinon, hexaconazole, 2,4-D quinalphos, carbofuran, validamycin, iprobenfos (ipropenphos), carbendazim, tricyclazole, imidacloprid, propiconazole, thiosultap-sodium (nerisoxin/dimeho), fenitrothion, chlorpyrifos, fthalide glyphosate, zineb, fipronil, tricyclazole, mancozeb, permethrin, bispyribac-sodium, fenobucarb, endosulfan, cypermethrin, benomyl, butachlor	Skin irritation, headache, dizziness, eye irritation, shortness of breath, acetyl cholinesterase inhibition	Vietnam; Dasgupta et al. 2007	9	Occupational (agricultural)
8	Methyl parathion, monocrotophos, acephate, malathion, phorate, triazophos, quinalphos, chlorpyrifos, endosulfan, endrin, indoxycarb, cypermethrin, spinosad, imadocloprid	Acute poisoning led to death of 22.6% subjects	India; Rao et al. 2005	6	Incidental
9	Endosulfan	Reduced male reproductive development among exposed children	India; Saiyed et al. 2003	20	Occupational (agricultural)
10	Monocrotophos, phosphamidon, dichlorvos, oxydemeton methyl, malathion, endosulfan, methyl parathion, dimethoate or carbaryl	Respiratory problems, ocular problems, gastrointestinal problems, dermal problems, decreased acetyl cholinesterase activity, elevated malondialdehyde (MDA) levels	India; Kesava chandran et al. 2006a	5	Occupational (agricultural)
11	Acephate, chlordane, dimethoate, allethrin, pipermoyl butoxide transfluthrin, endosulfan, chlorpyrifos, profenofos, monocrotophos, carbofuran, cypermethrin, cyfluthrin	Decrease in RBC (red blood cell count), haemoglobin, increase in WBC (white blood cell count) with a large number of immature cells and diagnosed as Philadelphia negative chronic myeloid leukemia, childhood signs of mental retardation	India; Jamil et al. 2007	1-5	Occupational (agricultural)
12	Organophosphates, including fenthion and some pyrethroids	Cytogenetic damage	Pakistan; Bhalli et al. 2006	13	Occupational (agricultural)
13	pp'-DDE, pp'-DDD, pp'-DDT, op-DDT, DDT, and $\alpha,\beta,\gamma,\delta$ -HCH	Inhibited acetyl- and butyryl-cholinesterase activities and showed elevated higher MDA levels; respiratory morbidity, ocular problems, gastro intestinal and skin problems	India; Singh et al. 2007	5	Occupational (agricultural)

linked to pesticides, and the benefits of personal protective equipment (face masks with replaceable filters and rubber gloves), do not always use such measures. Cost, general lethargy, and the discomfort of wearing protective equipment are the main reasons given for not using such devices. Some “make shift” protection is used, such as wearing long-sleeved shirts or breathing through a cloth held around the nose; however, such measures offer little defense (Indra et al. 2007). A standard practice among pesticide sprayers in developing countries is to chew or smoke tobacco while spraying “to reduce the nauseating feeling”; this practice accentuates the magnitude of the health hazard. Another practice observed in developing countries is mixing and spraying pesticides that share a common active ingredient. This practice may produce a more dangerous concoction, because mixing of pesticides may alter chemical properties, result in potentiation, and thereby increase detrimental effects (Salameh et al. 2004).

Farmers are also occupationally exposed to several potentially harmful environmental agents other than pesticides. Such agents include fertilizers, fuels, engine exhaust, organic and inorganic dusts, solvents, ultraviolet light, and zoonotic bacteria and viruses. Figures 1–6 visually depict farm practices associated with pesticide handling and spraying in India. Such farm scenes that depict different but common pesticide handling activities may provide visual evidence to the reader of the risks routinely faced by farmers in developing countries.



**Fig. 1** Spraying equipment on tractor used in North India





**Fig. 2** Pesticide application in a mango plantation, performed by a sprayer being assisted by a child



**Fig. 3** Lung function test performed on a pesticide sprayer by a researcher



**Fig. 4** Spraying pesticides without protective clothing or equipment



**Fig. 5** Filling a spray tank with pesticide spray solution



**Fig. 6** Mixing of pesticides with bare hands

## **4 Pesticides Used in Developing Countries**

A hazard categorization (WHO 2004) of insecticides and one herbicide (atrazine) commonly used in third world countries is presented in Table 1. The Food and Agriculture Organization (FAO) and WHO recommend that Ia (extremely hazardous) and Ib (highly hazardous) pesticides should not be used in developing countries (PAN UK 2001). The FAO/WHO also suggest that class II (moderately hazardous) pesticides should be avoided in those countries. Notwithstanding these recommendations, the practice of spraying such “powerful” pesticides continues (Table 2). Large chemical companies reinforce the widespread usage of pesticides in the farm sector by adopting aggressive marketing strategies that claim more potent pesticides are necessary to prevent crop loss (Nigg et al. 1990).

Literature searches reveal a few reports on the general health effects of pesticides in farming populations of developing countries; these, along with health surveys conducted by Indian Institute of Toxicology Research (formerly Industrial Toxicology Research Centre), Lucknow, India, during 1990–2007, are reviewed in the sections below.

## **5 Health Impact of Pesticides**

### **5.1 Neurological Effects**

The brain and peripheral nervous system are directly affected by pesticides, both as sites of action and deposition. All pesticide classes may affect brain and neural tissue, even if they do not cause observable effects. In practice, the pesticides that most

often affect the nervous system are the OP and *N*-methyl carbamate insecticides; these are responsible for most acute human poisonings in developing countries. In addition to acute poisoning, OPs may produce subacute, delayed and chronic neurological, neurobehavioral and psychiatric syndromes. Evidence for such chronic neurological and psychiatric effects of OP compounds come from case reports, clusters of neurological diseases, and from studies of exposed workers and other populations (Moses 1995).

OP compounds inactivate acetylcholinesterase by alkyl phosphorylation of a serine hydroxyl group at the esteratic site of the enzyme. The phosphorylated enzyme is inactive, and thus unable to hydrolyze acetylcholine (Karalliedde 1999), which causes transmission of nerve impulses to slow or stop.

Studies on neurological symptoms among Sri Lankan farmers show that 24% of acute pesticide poisoning cases result from occupational exposures to acetylcholinesterase-inhibiting insecticides. Exposed farmers showed significantly more inhibition of cholinesterase activity than did unexposed controls (Lydian et al. 2003). Higher nervous functions such as memory, learning, and vigilance were also found to be affected in subjects exposed to quinalphos (Srivastava et al. 2000).

In another study, a dose–effect relationship was found between the incidence of neurobehavioral symptoms and the use of multiple organochlorine insecticides (Kishi et al. 1995).

In an earlier Sri Lankan study (Peiris-John et al. 2002) on nerve and neuromuscular-function effects of occupational exposure to OP pesticides, reduced sensory conduction- and motor conduction-velocity was reported in farmers between cultivation seasons. Evidence of sensory dysfunction, after acute exposure, and sensory and motor impairment from long-term low-level exposure to OPs were observed in the study. Chronic neurological effects associated with pesticide exposure included slowing of nerve conduction velocity, slowing of reaction time, slowing of motor/visual speed tests, poorer performance on learning/memory tasks, impaired vibrotactile sensitivity, abnormal postural sway, increased beta activity of ECGs, decreased amplitude in visual evoked potentials, and decreased muscle strength. Other neurological effects potentially associated with pesticide exposure are amyotrophic lateral sclerosis, eye disorders, Guillian-Barre syndrome, movement disorders, multiple system atrophy, psychiatric disorders, and reflex sympathetic dystrophy (Moses 1995). About 3% of pesticide-exposed children showed signs of mental retardation and delayed milestones (Jamil et al. 2007).

Le Couteur et al. (1999) suggested that pesticide exposure may be associated with increased risk of neurodegenerative disease, particularly Parkinson's disease (PD). In cross sectional studies, an association was found between exposure to pesticides and PD, although no specific responsible pesticides or their classes were identified (Le Couteur et al. 1999). Several studies have implicated the herbicide paraquat (Liou et al. 1997), which produces selective degeneration of neurons and Parkinsonian-type effects. Case reports have described PD symptoms in individuals exposed to OPs (Bhatt et al. 1999), to herbicides, including glyphosate (Barbosa et al. 2001) and diquat (Sechi et al. 1992), and fungicides, including maneb (Meco et al. 1994). In severe cases of poisoning by OPs, such as chlorpyrifos, fenthion, malathion, and trichlorfon, muscle weakness, ataxia, and paralysis may occur after a period of apparent recovery. This condition is called OP-induced delayed neuropathy

and is characterized by axon degeneration and degeneration of myelin in the peripheral and central nervous system. Such delayed neurotoxic effects result from binding (phosphorylation) of a specific enzyme in nervous tissue called the neurotoxic esterase, rather than inhibition of cholinesterase (Moses 1995).

## **5.2 Respiratory Effects**

Health problems reported from farm workers using pesticides in Ethiopian fields showed respiratory symptoms such as coughing, phlegm, and wheezing (Ejigu and Mekonnen 2005). Insecticides, primarily those that inhibit cholinesterase, may cause respiratory symptoms among agricultural workers (Yemaneberhan et al. 1997; Ohayo-Mitoko et al. 2000). The prevalence of mild, moderate, and severe airway obstruction was observed in one study among pesticide sprayers (Kesavachandran et al. 2006a). The high respiratory morbidity may be attributed to high prevalence of smoking and prolonged inhalation of organic dusts during farming operations; such effects are associated with illiteracy and poor socioeconomic status (Gupta et al. 1995).

## **5.3 Dermal Effects**

Among 815 pesticide-induced injury cases (including poisoning) registered in Japan in 1968–1970, allergic contact dermatitis was diagnosed in 274 cases (33.6%) (Matsushita et al. 1980). Among 122 Taiwanese fruit farmers, who were spraying pesticides regularly, contact allergy to pesticides was found in 40%, and clinical symptoms of contact dermatitis in 30% of cases (Guo et al. 1996). In Spain, the prevalence of contact allergy to mercury and carbamates, which are components of many pesticide products, was three times as high among farmers, compared with the control group (Garcia-Perez et al. 1984). Among 104 Polish farmers, treated in a dermatology clinic for eczema, contact allergy to pesticides was found in two persons (Spiewak 2001). Among 263 hop growers in eastern Poland, contact allergy to pesticides was found in 66 persons (25.1%) (Spiewak 2001). Contact dermatitis leads to skin irritation in pesticide exposed subjects (Li 1986; Lisi et al. 1987). Clinical manifestation observed among pesticide sprayers (Nagami et al. 2005), in Japan, included acute dermatitis (24% of cases) and chemical burns (15% of cases).

## **5.4 Reproductive Abnormalities**

Data on reproductive toxicity, collected from couples engaged in spraying organochlorine, organophosphorus, and carbamate insecticides in cotton fields, show



**Table 3** A comparison of reproductive effects observed in couples engaged in spraying OC, OP, and carbamate insecticides (exposed column) in cotton fields, with unexposed subjects (Rupa et al. 1991)

Reproductive problems	Exposed (%)	Unexposed (%)
Abortions	26	15
Still births	8.7	2.6
Neonatal deaths	9.2	2.2
Congenital defects	3	0.1

abnormal reproductive performance (Table 3) (Rupa et al. 1991). A study (Saiyed et al. 2003) on endosulfan exposure in male children revealed a delay in sexual maturity and interferences with sex hormone synthesis.

## 5.5 Cancer

Farmers have a higher risk of stomach cancer, when compared with the general population, with a difference ranging from 1.05 to 1.12 (Acquavella et al. 1998; Blair et al. 1992; Meyer et al. 2003). Several studies have given indications that farmers or agricultural workers may have an excess brain cancer risk (Blair et al. 1992; Wingren and Axelson 1992; Brownson et al. 1990; Reif et al. 1989; Musicco et al. 1982). The association between environmental agents and brain cancer has not been fully evaluated, although several pesticides have demonstrated such carcinogenic potential in animal bioassays (Blair and Zahm 1993; IARC 1987; Hoover and Blair 1991).

The International Agency for Research on Cancer (IARC) has issued reports concerning the increased risk of developing skin and lip cancer among professional pesticide sprayers (IARC Working Group 1991). Strong carcinogenic properties were attributed to arsenical pesticides (Axelson 1987). Moreover, in a study conducted in Costa Rica, excess skin cancers (lip cancer, melanoma, nonmelanocytic skin and penile cancer) occurred in coffee growing areas, where paraquat and lead arsenate were extensively used (Wesseling et al. 1999).

## 5.6 Effects on General Health

Liver function tests showed elevated values of alkaline phosphatase, glutamate pyruvate transaminase, and glutamate oxaloacetate transaminase among sprayers of pesticides (Ejigu and Mekonnen 2005). Workers, who sprayed methomyl, a carbamate insecticide, showed significant changes in electrocardiography, indicating

cardiotoxic effects of this insecticide (Saiyed et al. 1992). Jamil et al. (2007) conducted epidemiological studies in 200 pesticide-exposed agricultural workers, and compared this population with an equal number of age- and sex-matched controls. Approximately 3% of subjects showed a decrease in red blood count, hemoglobin, and increases in white blood count, with a large number of immature cells.

## **6 Agrarian Health Surveys Conducted by IITR**

A health study conducted in Lucknow by the Indian Institute of Toxicology Research (ITRC 1990) on pesticide applicators (spraying a mixture of pesticides) working in Malihabad mango plantations, showed overall morbidity rates of 42.8%. The chief morbidities were respiratory disorders (33.4%), musculoskeletal disorders (15%), and those pertaining to the central nervous system (6%), predominantly peripheral neuropathy. Singh et al. (2007) and Kesavachandran et al. (2006b) performed studies to determine pesticide-exposure-related health problems among sprayers in mango orchards, and observed the following: respiratory morbidity (32.4%), ocular problems (8.8%), gastrointestinal effects (17.6%) and skin problems (23.5%) as well as inhibition of acetylcholinesterase and butylcholineesterase activities; higher malondialdehyde levels were also found among sprayers compared with controls. Srivastava et al. (1995) examined serum levels of thyroxin and thyroid stimulating hormone, with respect to blood levels of organochlorine insecticides, and observed depleted thyroxine levels in 24.3% subjects. Lung function abnormalities, especially prevalence of mild and moderate types of bronchial obstruction among sprayers were observed in earlier report (Rastogi et al. 1989).

## **7 Preventive Strategies**

Several legislative actions have been taken to regulate pesticide use in developing countries (Table 4) (FAO 2005). In India, the Insecticide Act (of 1968) and Insecticide rules (of) 1971, administered by the Ministry of Agriculture, regulates the import, manufacture, sale, transport, distribution, and use of insecticides, with the intent of preventing risk to human beings or animals and other matters connected therewith (JPC Report 2004). The Central Insecticide Board constituted under section 4 of this act advises Central and State Governments of India on technical matters, viz., (1) specifies safety measures necessary to prevent risk to human beings or animals in manufacture, sale, storage, distribution, and use, (2) assesses suitability for aerial application, (3) sets specifications for shelf life, (4) advises on residue tolerance limits and waiting periods, (5) recommends inclusion of chemicals/substances in the schedule of insecticide, and (6) other functions incidental to these matters (JPC Report 2004).

Effective enforcement of these laws can prevent public health problems and reduce environmental incidents with pesticides in developing countries.



**Table 4** A review of selected regulations and legislative acts for regulating pesticide use in developing countries

Country	Regulations/legislative acts
India	Insecticides Act (1968); Insecticides Rule (1971); Environmental Protection Act (1986); Prevention of Food Adulteration Act (1954)
China	China Pesticide Management Byelaw; China Pesticide Produce Regulation; China Production Quality Management Law
Malaysia	Pesticide Act (1974) and its amendment (2004); Occupational Safety and Health (1974); Environmental Quality Act (1974). Food Act (1983); Hydrogen Cyanide (Fumigation) Act (1956); Sodium Arsenate Regulations (1949)
Philippines	Presidential Degree No. 114; Letter of Instruction No. 986; Magna Carta Act for Small farmers; Consumer Act of the Philippines
Costa Rica	Law for the Control of Pesticides (1979); Law Governing Occupational Health (1981)
Mexico	Law on Plant and Animal Protection (1940)
Kenya	Pesticide Control Board Act (1982)
Bangladesh	Pesticide Ordinance (1971); Amendment to Pesticide Ordinance (1985); Pesticide Rules (1985)
Indonesia	Ministry of Agriculture Decree No. 439.1 of 2001; Ministry of Agriculture Decree No. 517 of 2002

Improved safety may also be achieved by reducing the quantity of pesticides used in agriculture. This can be achieved either through restricting the concentration of formulations or by increasing the dilution of the spray stream by increasing recommended water spray volumes (Indra et al. 2007). There is also ample scope for reducing pesticide exposure to applicators through creation of improved pesticide toxicity awareness programs. Such programs are consistent with integrated pest management, an eco-friendly approach for pest management that encompasses cultural, mechanical, biological methods and need-based use of chemical pesticides, with preference given to the use of bio-pesticides and bio-control agents. The use of integrated pest management has presumably led to a reduction in consumption of chemical pesticides from 65,462 t during 1994–1995 to 47,020 t during 2001–2002 in India (JPC Report 2004).

The existing welfare fund board for agricultural laborers could institute a special program for pesticide applicators, including health insurance protection measures. An extension strategy focusing on a program to improve awareness on the toxicity of pesticides, combined with provision of a subsidy for protective gear, alone, would result in health improvement among pesticide applicators (Indra et al. 2007). Considering the economic cost of pesticide use in developing countries, it makes economic sense to invest money to improve the safety of pesticide use, including provision of protective gear. Pesticide users would benefit from the State Department of Agriculture initiating programs with this particular objective (Indra et al. 2007).

Finally, agriculture should be declared an industry, and agricultural workers should be treated as industrial workers. They should have access to occupational health services, including health surveillance and exposure assessment, especially with respect to pesticide usage.

## 8 Summary

Developing countries use only 20% of the world's agrochemicals, yet they suffer 99% of deaths from pesticide poisoning. Pesticide poisoning is a significant problem in developing countries primarily because of unsafe pesticide application and handling practices. Safety is further exacerbated by the illiteracy and poverty that prevails in most farming communities of developing countries. Pesticides classified as being extremely or highly hazardous by FAO and WHO, including those banned by other countries, continue to be used in developing countries. Many farmers in developing countries continue to be exposed to pesticides from either storing them in or near their residences, or from inadequate or unsafe application or handling practices.

Farming populations exposed to pesticides suffer from several health problems, primarily neurological abnormalities, respiratory ailments, and reproductive, endocrinological, and dermal problems. In developing countries, the scientific literature that addresses adverse health effects of pesticides is scanty. Few research groups (including the Indian Institute of Toxicology Research, India) have taken the initiative to monitor health problems resulting from pesticide exposure in agrarian communities. The welfare fund for agricultural laborers could institute a special program for pesticide applicators in developing countries. The primary need, currently, in such countries is creation and implementation of sound national policies to effectively articulate appropriate guidelines for managing farm pest control activities. Such policies should be aimed at both limiting pesticide exposure and usage, but doing so without damaging the yields of food production. If such steps are taken, it is fully expected that the incidence of adverse health consequences for agrarian populations from pesticide toxicity will decrease, and the health of farmers improve.

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# Procedures for Analysis of Atrazine and Simazine in Environmental Matrices

Hanna Barchańska and Irena Baranowska

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## 1 Introduction

The triazine herbicides, particularly atrazine and simazine, are among the most popular groups of pesticides. A great majority of the triazine herbicides are derived from the *s*-triazine structure, a six-membered heterocycle with symmetrically located nitrogen atoms that are substituted at the 2, 4, and 6 ring positions. Table 1 contains a list of abbreviations and acronyms used in this article. Table 2 presents a listing of the chemical names, designations and CAS numbers for members of the triazine herbicide class.

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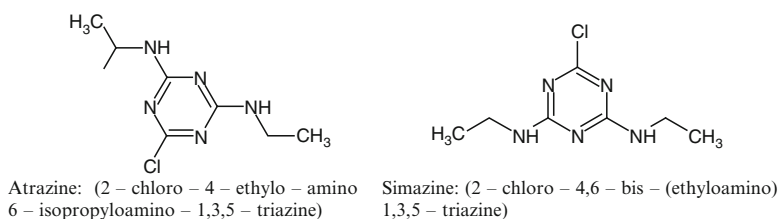


**Table 1** Abbreviations and acronyms used in this article

Abbreviations/Acronyms	Explanation
ASE	Accelerated solvent extraction
CE	Capillary electrophoresis
CW-DVB	Carbowax-divinylbenzene
CZE	Capillary zone electrophoresis
C18	18 Carbon (octadecyl) alkane bounded Silica packing
d	Days
DAD	Diode array detector
DB	Dibenzene
DBM	Dibuthylmelamine
DVB	Divinylbenzene
2D-TLC	Two-dimensional thin layer chromatography
ECD	Electron capture detector
ELISA	Enzyme-linked immunosorbent assay
FL	Fluorescence detector
FID	Flame ionization detector
FPD	Flame photometric detector
GC	Gas chromatography
HPLC	High-performance liquid chromatography
LC	Liquid chromatography
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
MAE	Microwave-assisted extraction
MEKC	Micellar electrokinetic chromatography
MIP	Molecular imprinted polymers
MS	Mass spectrometry
MSPD	Matrix solid-phase dispersion
NPD	Nitrogen-phosphorus detector
n.r.	Not reported
OV	Organic vapor
p	Pressure
PDMS	Polydimethylsiloxane
RP18	C18
SAX	Strong anion exchanger
SCX	Strong cation exchanger
SDB	Copolymer styrene - divinylbenzene
SE	Size exclusion
SFE	Supercritical fluid extraction
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SRMM	Online strategies, sweeping and stacking with reverse migration micelles
T	Temperature
t	Time
THF	Tetrahydrofuran
TLC	Thin layer chromatography
UAE	Ultrasound-assisted extraction
UV	Ultraviolet

**Table 2** Common and chemical names and CAS numbers for various members of the triazine or triazinone (metribuzin and hexazinone) herbicide classes

Trade name	Chemical name	Chemical designation	CAS
Ametryn	2-Ethylamino-4-isopropylamino-6-methylthio-1,3,5-triazine	Herbicide	834-12-8
Atrazine	2-Chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine	Herbicide	1912-24-9
Cyanazine	2-[(4-Chloro-6-ethylamino-1,3,5-triazin-2-yl)amino]-2-methylpropanenitrile	Herbicide	21725-46-2
Desethylatrazine	2-Amino-4-chloro-6-isopropylamino-1,3,5-triazine	Herbicide	6190-65-4
Desisopropylatrazine	2-Amino-4-chloro-6-ethylamino-1,3,5-triazine	Herbicide	1007-28-9
Hexazinone	3-Cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4-dione	Herbicide	51235-04-2
Metribuzin	4-Amino-6- <i>tert</i> -butyl-3-methylsulfanyl-1,2,4-triazin-5-one	Herbicide	21087-64-9
Prometon	2,4-Bis-(isopropylamino)-6-methoxy-1,3,5-triazine	Herbicide	1610-18-0
Prometryn	2,4-Bis-(isopropylamino)-6-methylthio-1,3,5-triazine	Herbicide	7287-19-6
Propazine	2,4-Bis(isopropylamino)-6-chloro-1,3,5-triazine	Herbicide	139-40-2
Simazine	2,4-Bis(ethylamino)-6-chloro-1,3,5-triazine	Herbicide	122-34-9
Terbutylazine	4-( <i>tert</i> -Butylamino)-6-ethylamino-5H-1,3,5-triazin-2-one	Herbicide	66753-07-9
Terbutryn	<i>N-tert</i> -Butyl- <i>N'</i> -ethyl-6-methylsulfanyl-1,3,5-triazine-2,4-diamine	Herbicide	886-50-0

**Fig. 1** Structures of atrazine and simazine

The *s*-triazines are stereochemically stable and certain of their degradation products are environmentally persistent, remaining in soil after application for several months to many years (Pacácková et al. 1996). The structures for atrazine and simazine, and an overview of their physical and chemical properties, are presented in Fig. 1 and Table 3, respectively.

Atrazine and simazine herbicides are readily absorbed by plant roots. After entering plants, these herbicides act by interfering with the enzyme systems responsible for the photolysis of water, thereby halting photosynthesis. Triazines are effective as pre- and postemergence herbicides for broad spectrum control of annual and perennial grasses and annual broad-leaved weeds (Dean et al. 1996).

**Table 3** Physical and chemical properties of atrazine and simazine

	Atrazine	Simazine	Reference
Solubility in water (mg L <sup>-1</sup> , 22°C, pH 7)	33	6.2	Garcinuno et al. 2003
log $K_{ow}$ (25°C)	2.5	2.1	
Absorption maxima and corresponding absorption coefficients	$\lambda = 222$ nm, $\epsilon = 41,000$	$\lambda = 222$ nm, $\epsilon = 36,000$	Pacácková et al. 1996
	$\lambda = 263$ nm, $\epsilon = 3,900$	$\lambda = 263$ nm, $\epsilon = 3,100$	
Soil sorption constant $K_{oc}$	100	130	Andreu and Picó 2004
Melting point (°C)	171-174	226-227	Dean et al. 1996
Vapor pressure (20°C) $\times 10^6$ mmHg	0.3	0.0061	
LD <sub>50</sub>	3,080 (rats)	5000 (rats)	
pK <sub>a</sub>	1.70	1.62	Pinto and Jardim 2000
Half-life in soil (d)	60	60	Andreu and Picó 2004

Analytical methods are needed for all pesticides, not only to trace environmental behavior, but also to ensure, through routine or special monitoring activities, that the amounts of applied compounds or their metabolites do not exceed safe levels as established by various governmental authorities. In addition, procedures are needed for the analytical determination of triazines in environmental samples to:

1. design and improve effective extraction approaches for determining trace levels in multiple sample types (Meakins et al. 1996; Ting and Tamashiro 1996; Mendaš et al. 2001; Xiong et al. 1998; Matsui et al. 2000; Kim et al. 2003),
2. investigate the behavior of triazines in the aqueous or other environments (Topp et al. 1995; Meakins et al. 1996; Funari et al. 1998; Herwig et al. 2001; Katz et al. 2001; Lesan and Bahandri 2003; Barriuso et al. 2004; Ying et al. 2005), soil (Houot et al. 2000; Abate et al. 2004; Xu et al. 2002), and to
3. establish degradation mechanisms, levels of sorption, and stability in the environment (Lanyi and Dinya 2003; Prosen and Zupančič-Kralj 2005).

## 2 Extraction and Preparation of Environmental Samples for Triazine Analysis

Figure 2 is a schematic conceived by the authors that depicts the general approach by which triazine herbicide products and their metabolites are analyzed in various environmental samples (Barchańska 2007).

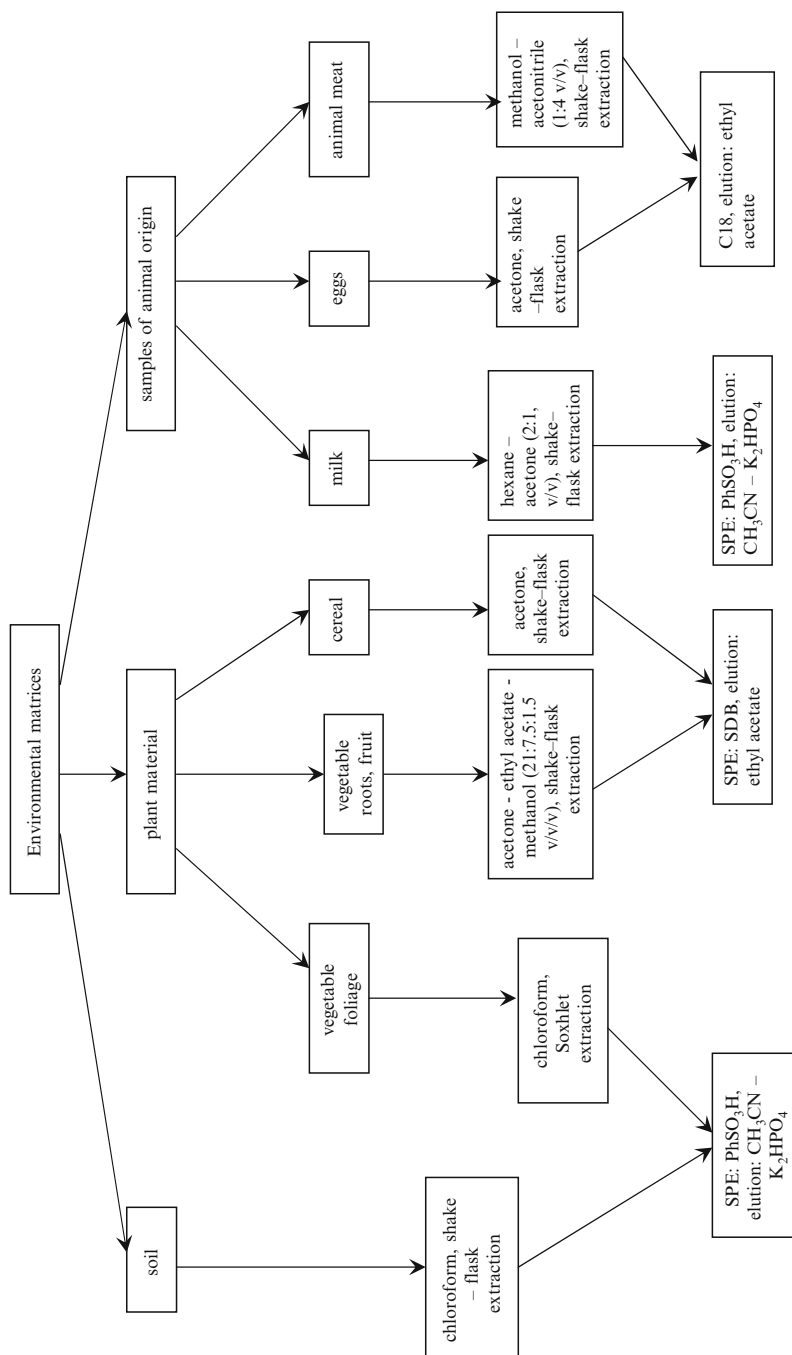


Fig. 2 Procedures for atrazine and simazine determination in trophic chain samples (Barchańska 2007)

## 2.1 Soil

Atrazine and simazine are usually extracted from soil samples by means of shake-flask extraction, ultrasound-assisted extraction and microwave-assisted extraction (MAE). Supercritical fluid extraction (SFE) is also available and is becoming increasingly popular. The conditions under which triazines are extracted from soil samples are summarized in Table 4.

Shake-flask extraction is often preferred for extraction of atrazine and simazine from soil samples, because it is simple, cheap, and achieves high recovery. Organic solvents and water are used as the extracting agents (Ying et al. 2005). Nevertheless, large volumes of solvents are required with the shake-flask method, and extracts have to be filtered and subjected to additional clean-up procedures before analysis.

Soxhlet extraction is also a routine procedure of choice in many laboratories. This extraction approach provides high recoveries (>60%), and filtration of extracts is unnecessary. The disadvantage of this technique is that large volumes of solvents (100–500 ml) are used and an additional clean-up step is usually necessary. Furthermore, soxhlet extraction is time-consuming and presents a risk of decomposition from the high temperature to which analytes are exposed during the extraction procedure.

Ultrasound-assisted extraction is also a cheap and effective extraction technique. It is conducted at room temperature and at atmospheric pressure. Such standard conditions prevent analytes from decomposition; nevertheless, the duration of the extraction procedure should be matched to the character of matrices and analytes under analysis, because excessively long extractions may accentuate triazine decomposition (Babić et al. 1998). In summary, use of ultrasound-assisted extraction with the proper extracting solvents provides high recoveries; however, the method is time-consuming and requires additional clean-up prior to analysis.

SFE is another effective extraction approach, and is becoming increasingly popular. It is used as an alternative to shake-flask and Soxhlet extraction techniques (Namieśnik et al. 2000). SFE provides high recovery rates, and extracts do not contain excessive amounts of interfering matrix compounds, reducing the need for extract clean-up. The drawback for SFE is that it requires expensive apparatus (Pacáková et al. 1996; Kreuzig et al. 2000; Motohashi et al. 2000; Richter et al. 2003).

A newer approach by which soil samples are extracted for triazine analysis is by means of MAE. The main advantage afforded by MAE is reduction of extraction time. Additionally, MAE reduces consumption of organic solvents and is amenable to running multiple samples concurrently (Namieśnik et al. 2000; Sparr Eskilsson and Bjorklund 2000). MAE extraction may be performed in either open- or closed-vessel systems (Falqui-Cao et al. 2001). The drawback of this technique is high cost of the equipment.

Another approach used to extract triazines from soils is the accelerated solvent extraction approach. This approach enables fast and effective extraction of triazines from soil samples. Analytes are extracted from the matrix with a solvent or solvent mixture at a preselected temperature and pressure. The most popular solvent is

**Table 4** A survey of conditions under which triazines are extracted from soil samples

Compound	Type of extraction	Solvent	Conditions of extraction	Clean up of extract	Recovery (%)	Comment	References
Atrazine, metribuzin, alachlor	Shake-flask extraction	80% Methanol	T = 20°C and T = 60°C	SPE	20–50	Higher recoveries were obtained during extraction at 60°C	Olness et al. 2002
Atrazine, simazine	Shake-flask extraction	Methanol, 0.01 M water solution of CaCl <sub>2</sub> , 0.01 M solution of CaCl <sub>2</sub> in water-methanol (8:2 v/v)	–	SPE: C18; elution: water-methanol	72	Recovery depended on type of soil. The influence of <i>Pseudomonas</i> sp. strain ADP on mineralization of atrazine was also investigated	Barriuso and Houot 1996; Farenthorst et al. 2000; Baran and Oleszczuk 2003; Barriuso et al. 2004
Atrazine, simazine	Shake-flask extraction	Water, methanol (1,2,3,5,10,90%), Triton-X 100, Tween 20, Tween 80, 3–40 mg L <sup>-1</sup>	(1) Solvents were aspirated through columns, where soil samples were placed, (2) shake-flask extraction	–	95	Concentrations of methanol above 10% did not increase the recovery; addition of surfactants increased the recovery	Ying et al. 2005
Atrazine, simazine, metabolites	Shake-flask extraction	Acetonitrile-methanol-water (7:1.5:1.5 v/v/v), next dichloromethane-water (1:2) <i>n</i> -hexane-acetone (1:1 v/v)	–	SPE: Al <sub>2</sub> O <sub>3</sub> elution: methanol	–	Triazine mobility in soil was investigated	Meakins et al. 1996
Atrazine, simazine	Shake-flask extraction	<i>n</i> -hexane-acetone (1:1 v/v)	–	SPE: graphitized carbon	87 (atrazine), 91 (simazine)	–	Funari et al. 1998
Atrazine, simazine	Shake-flask extraction	Methanol-water (10:1 v/v)	2 hr	SPE: C18; elution: methanol	89 (atrazine), 95 (simazine)	Different solvents and types of extraction of triazines from trophic chain samples were investigated. The highest recovery was obtained by means of shake-flask extraction with methanol-water (10:1 v/v)	Baranowska et al. 2006

(continued)

Table 4 (continued)

Compound	Type of extraction	Solvent	Conditions of extraction	Clean up of extract	Recovery (%)	Comment	References
Triazines	Shake-flask extraction	Acetonitrile-water (9:1 v/v), acetone- <i>n</i> -hexane (1:1 v/v), ethyl acetate	n.r.	SPE: Al <sub>2</sub> O <sub>3</sub> and Florisil	n.r.	Review article	Tadeo et al. 1996
Bromacil, hexazinone, metoxuron, propazine, simazine	Shake-flask extraction	Chloroform	n.r.	SPE: C18	95–102	Conditions for atrazine extraction from soil samples containing other groups of herbicides and phenols were worked out	Baranowska and Pieszko 2000a
Atrazine, simazine	SFE, shake-flask extraction	CO <sub>2</sub> , methanol-modified CO <sub>2</sub> , 5% methanol	70 min SFE	–	63	–	Dean et al. 1996; Kreuzig et al. 2000
atrazine, simazine	SFE	CO <sub>2</sub> , CO <sub>2</sub> -water-methanol, water-acetone, acetone-water-methanolamine	T = 80°C, p = 400 bar, t = 30 min	–	79–119	Max. recovery: CO <sub>2</sub> + 5% methanol; min. recovery: CO <sub>2</sub> -acetone-water-methanolamine	Camel 1997
Atrazine, simazine	SFE	CO <sub>2</sub> modified with methanol and acetone	T = 50°C, p = 50 MPa	–	120	Influence of pressure, temperature, and volume of modifier was investigated	van der Velde et al. 1994
Atrazine	SFE	CO <sub>2</sub> modified with methanol	50 MPa, T = 50°C, 100 µl/cell	–	90	Extraction techniques were compared	Pacáková et al. 1996; Richter et al. 2003
Atrazine, simazine	Soxhlet extraction	Methanol	t = 2 hr	–	93	–	–
Atrazine, simazine	Subcritical water extraction	Water	T = 90°C, t = 5 min	–	81–93	Extraction techniques were compared	Motohashi et al. 2000
Atrazine, simazine	Soxhlet extraction	Methylene chloride-acetone (1:1 v/v)	n.r.	SPE: Carbo graph 4	–	–	–



Atrazine and meta-bolites, prometryn, simazine	MAE	Acetone-hexane (1:1 v/v)	T = 115°C, t = 20 min	SPE: C18, silicagel,	75-100	Closed-vessel system	Dean and Xiong 2000; Sparr Eskilsson and Björklund 2000
	MAE	Toluene-water (10:1 v/v)	t = 6 min, power 600 W	ion-exchange resin	97-102	Open-vessel system	
Atrazine, simazine, metribuzin, terbutylazine	MAE	Water, acetonitrile	t = 2-5 min, T = 80±105°C	SPE: CW-DVB	87	Review article	Andreu and Picó 2004
Triazines	MAE	Water, acetonitrile-0.5 ammonium solution (7:3 v/v), dichloromethane-water (1:1 v/v), dichloromethane-methanol (9:1 v/v)	T = 100°C, power 950 W, t = 20 min	n.r.	97-100	The highest recovery was obtained with a mixture of dichloromethane-methanol (9:1 v/v)	Camel 2000
Atrazine, simazine, propazine	MAE	Water, water-acetone, water-methanol	t = 1-20 min, T = 85-150°C	SPME (CW-DVB)	76.1-87.2	Max. recovery: t = 3 min, T = 105°C, water-methanol	Gang and Lee 2003
Atrazine, simazine	MAE	Water, dichloromethane, HCl, acetone- <i>n</i> -hexane, ammonium ammonium chloride (pH 9.8), acetic acid-sodium acetate (pH 4.7)	t = 4 min, power 600 W, 30 ml of solvent	n.r.	87.7-99.8 (atrazine); 88.5-100.4 (simazine)	The highest recovery was obtained with methanol	Xiong et al. 1998

(continued)

Table 4 (continued)

Compound	Type of extraction	Solvent	Conditions of extraction	Clean up of extract	Recovery (%)	Comment	References
Atrazine, desethylatrazine, desisopropyl atrazine, simazine	MAE	HCl, pH 1.0, pH 4.5, water, solution of ammonium and ammonium chloride, pH 9.2, ammonium solution pH 11.1	t = 3–5 min, p = 0.1–0.5 MPa, power 600 W	n.r.	85.6–91.5	The highest recovery: t = 3 min, solvent – water	Sparr Eskilsson and Björklund 2000
Atrazine, simazine	MAE	Acetone, dichloro-methane, methanol, <i>n</i> -hexane, water	n.r.	n.r.	n.r.	–	Singh et al. 2004
Atrazine and metabolites, simazine, prometryn	ASE	Methanol	p = 10 MPa, T = 125°C, t = 10 min	–	47–99	Review article	Dean and Xiong 2000
Atrazine, simazine, metribuzin	ASE	Phosphate buffer, pH 7	T = 90°C	SPE: C18	65	Review article	Andreu and Picó 2004
Atrazine	Ultrasonic solvent extraction	Acetone, acetonitrile, benzene, chloroform, diethyl ether, dichloro-methane, <i>n</i> -hexane	t = 15 min, 20 ml of solvent	n.r.	103–108	The highest recovery was obtained with acetone; recoveries obtained by ultrasonic solvent extraction and shake-flask extraction were comparable	Babić et al. 1998; Helaleh et al. 2005
	Soxhlet extraction		t = 4 hr, 250 ml of solvent				
	Shake-flask extra ction		t = 4 hr, 20 ml of solvent				

water. Water is effective as a solvent when extracting triazine residues from soil if the temperature and pressure are moderately high (Luque-Garcia and de Castro 2002; Andreu and Picó 2004). Extraction from many samples concurrently is possible when using AFE, because the duration of the extraction process is short (~156min) and can easily be automated (Andreu and Picó 2004).

## 2.2 *Vegetables and Fruits*

Procedures used for the extraction of triazines from vegetation samples are summarized in Table 5.

Several authors (Torres et al. 1996; Hogendoorn and van Zoonen 2000; Motohashi et al. 2000; Tadeo et al. 2000; Buldini et al. 2002) have described extraction procedures for processing plant samples when analyzing for a broad spectrum of chemical compounds (including the triazine herbicides). A review of such procedures indicates that organic solvents (acetone, acetonitrile, ethyl acetate, methanol, chloroform, etc.), solvent mixtures, and even buffer solutions are commonly used to extract triazine from vegetables, fruits, and cereals (Ahmed 2001). Ethyl acetate is often the recommended solvent for extracting more polar pesticides (phenylureas), whereas acetone is used to extract less polar pesticides (organochlorines) (van der Hoff 1996).

Matrix-solid phase dispersion is a method that has been applied to triazine herbicide extraction, and to other pesticides residues of plant origin, as well. This technique replaces the standard two-step procedure (solvent extraction and clean-up step) with a single-step process, in which the sample is mixed with a sorbent, and analytes are eluted from the sorbent with the appropriate solvent(s). Extractions of triazines from vegetables and fruits have frequently been performed using various organic solvents at ambient temperatures, or with heated solvents.

In summary, triazines are successfully extracted from vegetables, fruits, and cereals with several solvents or solvent mixtures, and are further processed using various clean-up techniques. In all cases high recoveries were obtained.

## 2.3 *Water*

The European Economic Community (EEC) policy (Directive no. 80-778)\EEC) fixes a low maximum concentration level for potable water ( $0.1 \mu\text{g L}^{-1}$ ) for each individual pesticide, and a collective limit ( $0.5 \mu\text{g L}^{-1}$ ) for all pesticides and related compounds. The concentration of atrazine and simazine in surface, ground, and tap water often exceeds the officially tolerated or legal level (Abgekodo et al. 1996). A review of the standard conditions under which triazines are commonly extracted from water is presented in Table 6.

**Table 5** Procedures for preparing vegetable and fruit samples for triazine analysis

Analyte	Sample	Type of preparation/ extraction	Solvent	Condition of extraction	Extract clean-up	Recovery (%)	Remarks	Reference
Atrazine	Apples	Grinding	Acetone-ethyl acetate-methanol (21:7.5:1.5, v/v/v)	n.r.	SPE: C18, elution: methanol	87-107	-	Liu and Lee 1998; Tadeo et al. 2000
Triazines	Carrots, peas, celery, apples, lemons, pears	Shaking	Acetonitrile-water, methylene chloride	12 hr	SPE: SCX	50-80	Simultaneous determination of fungicides, triazines, and insecticides	Batista et al. 1989; Schachterle and Feigel 1996
Simazine	Alfalfa, maize, beet, cucumbers, tomatoes	Shaking	Water, chloroform	75 g of sample	SPE: Al <sub>2</sub> O <sub>3</sub> elution: hexane-benzene (1:1, v/v)	79-93	Accumulation of simazine by different species of plants was investigated	Pringle et al. 1978
Atrazine, simazine	Cereals	Shaking	Acetone	1 hr	SPE: C18, elution: methanol	71 (atrazine) 77 (simazine)	Different solvents and SPE sorbents were tested	Baranowska et al. 2005
Triazines	Carrots, celery, onions, potatoes, grapes, strawberries, cereals	Shaking	Methanol	n.r.	SPE: SAX	n.r.	Review article	Hogendoorn and van Zoonen 2000; Ahmed 2001
Atrazine, simazine	Green parts of vegetables	Soxhlet extraction	Chloroform	8 hr	SPE: PhSO <sub>3</sub> H, elution: acetonitrile: K <sub>2</sub> HPO <sub>4</sub> , 0.1 M (1:1, v/v)	87 (atrazine), 93 (simazine)	Different solvents and SPE sorbents were tested	Baranowska et al. 2006; Barchańska 2007
	Vegetables roots	Shake-flask extraction	Acetone	1 hr		86 (atrazine), 89 (simazine)		
	Strawberries, sweet cherries		Acetone-ethyl acetate-methanol (21:7.5:1.5 v/v/v)	1 hr	SPE: SDB, elution: ethyl acetate	92 (atrazine), 95 (simazine)		

Atrazine, linuron, carbaryl, propazine, simazine	Carrots	Sonication	Acetone-dichloromethane (1:1, v/v), addition of sodium chloride (4 g)	5 g of sample, t = 15 min, volume of solvent: 30 ml	SPE: amino modified octadecylsilica, elution dichloromethane-methanol (99:1, v/v)	n.r.	-	Babić et al. 1998; da Silva et al. 2003
Atrazine	Carrots, potatoes	Shaking	Acetonitrile	n.r.	SPE: C18	n.r.	-	Hogenboom et al. 1999
Atrazine, metribuzin, simazine	Currants	MSPD	Homogenization with Florisil, silica gel, elution: ethyl acetate			90-95	-	Torres et al. 1996; Tadeo et al. 2000
Atrazine	Apples, oranges, pea	Grinding	Acetone, methanol	SPE: Al <sub>2</sub> O <sub>3</sub> , Florisil, elution: ethyl acetate + 1% triethylamine, next ethyl acetate-acetone (9:1 v/v)		88-99	Similar procedure was described by Zupančić-Kralj 2003	Hock (1993) and Tadeo et al. (2000)
Simazine	Blueberry, fruit juices	Grinding	Acetonitrile	n.r.		-		Tadeo et al. 2000
Ametryn, atrazine, cyanazine, propazine, simazine, terbuthryn	Vegetables, rice	Grinding	Dichloromethane	Silica gel				
	Cereals, apples, celery	Grinding	Methanol	LLS, SPE: SCX				
	Vegetables	Grinding	Acetone	LLS, SPE: Florisil				
	Cereals, fruits, vegetables	Shaking	Methanol	Al <sub>2</sub> O <sub>3</sub>				
	Vegetables, rice		Acetone, dichloromethane, methanol	SPE: Florisil, SCX, Al <sub>2</sub> O <sub>3</sub>				
Atrazine	Fruits, vegetables	Shaking	Ethyl acetate	LLS - Florisil				
Simazine	Grape juice	LLE	Acetonitrile	n.r.				
	Olive oil	Grinding	Ethyl acetate	n.r.				
	Olives	Grinding		n.r.				
Atrazine, metribuzin, simazine	Sugar beat, celery, cereals	Shaking	Chloroform, dichloromethane, water, acetonitrile-water	SPE: silica gel, SAX, Al <sub>2</sub> O <sub>3</sub>				
		Soxhlet extraction						

(continued)

Table 5 (continued)

Analyte	Sample	Type of preparation/ extraction	Solvent	Condition of extraction	Extract clean-up	Recovery (%)	Remarks	Reference
Atrazine	Vegetables	ASE	Hexane-acetone-acetonitrile		T = 125°C, p = 10 MPa	n.r.	n.r.	Buldini et al. 2002
Atrazine, cyanazine, simazine	Vegetables, fruits	SFE	CO <sub>2</sub>	T = 50°C, p = 350 MPa, t = 23 min	-	80-96	Similar procedure was applied by Camel (1997), Lehotay (1997), Lehotay and Valverde-Garcia (1998)	Motohashi et al. 2000
Atrazine, simazine	Cereals	SFE	CO <sub>2</sub> + 10% of methanol	T = 60°C, p = 200 atm	-	62.6-99.8	-	-
Anetryn, atrazine, cyanazine, propazine, simazine	Oranges	MAE	Acetone- <i>n</i> -hexane (1:1 v/v)	n.r.	n.r.	n.r.	Review article	Tadeo et al. 2000
Atrazine and other pesticides	Olive oil	Ultrasound-assisted extraction	THF, ethyl acetate, acetonitrile	t = 15 min	-	94.5%	Review article	Tadeo et al. 2000; Bারেk et al. 2003
Atrazine, simazine	Olives, olive oil	MSPD	Samples were homogenized with sorbent Bondesil - NH <sub>3</sub>			81-111	-	Ferrer et al. 2005

**Table 6** Procedures commonly used to prepare water samples for triazine analysis

Analyte	Sample	Extraction conditions	Recovery (%)	Remarks	Reference
Atrazine, simazine	Ground water	SPE: C18, SDB, elution: methanol	98 (atrazine), 96 (simazine)	The highest recoveries were obtained by means of double SDB disks	Turiel et al. 2000
Atrazine and metabolites	Surface and ground water	SPE: C18, Oasis, LiChlorutEN, elution: methanol	95–100	The highest recoveries were obtained by means of polymeric sorbents. Similar extraction conditions were applied by Tadeo et al. (2000), Pinto and Jardim (2000), Picó et al. (2003)	Carabias-Martinez et al. 2002
Atrazine, metribuzin, prometryn, simazine, terbuthylazine	Surface water, rainfall	SPE: C18, elution: acetone/nitrite, ethyl acetate, methanol, methylene chloride; elution: acetone, LLE: dichloromethane	58.3–90.7 (SPE), 79.3–95.5 (LLE)	Different SPE sorbents were evaluated for the extraction of triazines, organochlorine, carbamates, and acidic pesticides. The recoveries obtained using C18 extraction columns and LLE were compared	Crespo et al. 1994; Volmer et al. 1994; Dean et al. 1996; Vassilakis et al. 1998
Atrazine, linuron, carbaryl, propazine, simazine	Drinking water	SPE: C18, elution: methanol	n.r.	SPE in combination with online strategies (SRMM) allowed the determination of pesticides at the level 0.1 µg L <sup>-1</sup> level in drinking water	da Silva et al. 2003
Atrazine, cyanazine, propazine, simazine, terbuthylazine	Surface water	SPE: DBM	97 (atrazine)	Triazine herbicides - selective polymer spheres were prepared by molecular imprinting using dibutylmelamine	Matsui et al. 2000
Simazine, propazine, hexazinone, metoxuron, bromacil	Surface water	SPE: PhSO <sub>3</sub> H, C18	98.9	The conditions under which herbicide were separated from water samples in the presence of phenols were determined	Baranowska and Pieszko 2000c
Triazines	Surface water and drinking water	SPE: SDB, elution acetone/nitrite-phosphate buffer (pH 7)	a > 95	–	Pichon and Hennion 1994; Pichon and Roguiaux 1999
Atrazine, propazine, simazine	Surface water and drinking water	SPME	84.3 (atrazine) 107.4 (propazine) 93.8 (simazine)	Amphiphilic and hydrophilic oligomers were synthesized and coated on fused silica capillaries using a so-gel technique. Sol-gel-coated capillaries were evaluated for the solid-phase microextraction and preconcentration of wide variety of analytes	Basheer et al. 2005
Atrazine	Surface water	LLE: water-chloroform	98.9	–	Kesari and Gupta 1998
Atrazine, simazine	Surface water	LLE: water + H <sub>2</sub> SO <sub>4</sub> dichloromethane	93–100	–	Durand et al. 1992

(continued)



Table 6 (continued)

Analyte	Sample	Extraction conditions	Recovery (%)	Remarks	Reference
Atrazine, simazine	Surface water	SPE: C18, elution: methanol, nanofiltration	50–100	The influence of natural organic matter on the removal of atrazine and simazine by nanofiltration was demonstrated	Agbekodo et al. 1996
Atrazine	Surface water	LLE: chloroform	n.r.	–	Topp et al. 1995
Atrazine, simazine	Surface water	LLE: methanol, acetone	84–95	Offline extraction coupled with precolumn derivatization liquid chromatography fluorescence detection procedure was developed	Gong and Ye 1998
Atrazine, simazine	Drinking water	LLE: water saturated with CO <sub>2</sub> -hexane	95–110	–	Pesando et al. 2004
Atrazine and metabolites, cyanazine, simazine	Drinking water	SPE: immunosorbents, activated carbon	>80	SPE on activated carbon sorbents enables concentrating pesticides of different polarities	Hogenboom et al. 1999; Borba da Cunha et al. 2004
Atrazine, simazine	Surface water and drinking water	SFE, CO <sub>2</sub> , CO <sub>2</sub> + methanol or acetone	107	Review article	Dean et al. 1996
Atrazine, propazine, simazine	Water rich in humic substances	SPE: MIP, elution: water	75	Review article	Hogendoorn and van Zoonen 2000

There are three common techniques used to extract triazines from water samples. The first one is solid-phase extraction (SPE). The SPE procedure relies on immunosorbents to trap the sought after analytes (Thomas et al. 1994; Pichon and Hennion 1994; Ferrer et al. 1997), and are effective for most triazine herbicides; SPE is also amenable for use in multiresidue analysis procedures (Pichon et al. 1999; Hogendoorn and van Zoonen 2000).

The second approach is liquid-liquid extraction. This technique is cheap and easy to conduct; however, it requires large solvent volumes. The last technique is the previously described SFE approach. It is believed to be environmentally friendly, because it does not require use of organic solvents; unfortunately, the SFE equipment is expensive. All of these techniques provide high recoveries; the selection of the appropriate extraction conditions and method often defaults to what laboratory equipment is available.

## ***2.4 Samples of Animal Origin***

Procedures commonly used for extracting triazines from animal tissues are summarized in Table 7.

Because triazines accumulate in fatty tissue, when analyzing samples of animal origin for triazines, it is first necessary to separate fat from other tissue types by selective extraction or melting (Ahmed 2001; da Silva et al. 2003). After fat is separated from other tissues, more common methods can be applied. The most popular extraction techniques for fat samples are SFE, MAE, and solid-phase microextraction (López-Avile et al. 1994; King and Zang 1998). Recently, the use of membranes for selective separation of organic compounds from animal tissues is becoming a useful alternative to the conventional procedures of liquid-liquid extraction and solid-liquid extraction. Moreover, such techniques can be coupled with most chromatographic systems (Jönsson and Mathiasson 1999; Carabias-Martinez et al. 2000). The most often used solvents for extracting triazines from animal tissues are alcohols, particularly methanol or a mixture of methanol with another organic solvent.

Triazines are usually extracted from liquid tissues (blood, urine) by means of SPE, or solid-phase microextraction techniques.

## ***2.5 Sample Preparation: Conclusion***

Determination of triazines in soil and trophic chain sample matrices usually involves working out different extraction procedures for different types of samples. This task is often complicated when sample matrices are complex.

We draw the following conclusions from the foregoing review of the analysis of triazines in soil and trophic samples:

**Table 7** Procedures for preparing samples of animal origin for triazine analysis

Analyte	Sample	Extraction methods	Solvent	Conditions of Recovery extraction (%)	Remarks	Reference
Atrazine	Beef kidney	ASE, SPME, MSPD: polyacryl resin	water-ethanol	T = 100°C, n.r. t = 10 min	Review article	Carabias-Martinez et al. 2005
Arazine	Tissues of fresh-water snails	homogenized with methanol, followed by ultrasonication Soxhlet extraction	Methanol	5 min 12 hr	The highest recoveries were obtained by the sonication. According to authors, sonication of biological material destroy cell membranes, hence increased the contact between the sample matrix and extraction solvent	Munoz and Roses 2000
Atrazine	Animals' kidney, liver	ultrasonication	Methanol-acetonitrile (1:4; 2:3 v/v)	30 min	A Comparison of two different extraction mixtures showed higher atrazine concentrations for methanol: acetonitrile (1:4 v/v)	Scutaru et al. 1998
Atrazine	Eggs	Soxhlet extraction, membrane separation	Hexane	120 min	The clean-up step using membrane filtration gave chromatograms with fewer interferences	Carabias-Martinez et al. 2000
Atrazine	Eggs	SFE	CO <sub>2</sub> + ethanol	T = 25°C, p = 27.6 MPa	Review article	Buldini et al. 2002
Atrazine	Meat	MAE	2-propanole	n.r. 40-90	Review article	Carabias-Martinez et al. 2000

Ametryn, atrazine, cyanazine, prometon, prometryn, propazine, simazine	Human blood plasma	SPE: Sep – Pak C18, Elution: chloroform–methanol (9:1, v/v), chloroform	n.r.	>60	SPME proved to be useful for extracting amino group-containing pesticides from biological samples	Kumazawa et al. 1992; Kumazawa and Suzuki 2000
Atrazine, simazine, organophosphorus, organochlorine, carbamate pesticides	Human blood plasma	SPE: Oasis sorbent	n.r.	>40	Polymeric-based sorbent Oasis cartridges were tested. This sorbent retained both apolar and polar compounds	Lacassie et al. 2001
Ametryn, atrazine, cyanazine, prometon, prometryn, propazine, simazine	Human blood, urine	SPE, fiber: PDMS, Acetone PDMS –DVB, CW–DVB	n.r.	21–99 (blood), 13.6–28.1 (urine)	The highest recoveries were obtained using PDMS fibers	Kumazawa and Suzuki 2000
Atrazine and metabolites, ametryn, prometryn, simazine	Human urine	SPE: SDB sorbent	n.r.	78–101	The SDB cartridge quantitatively retained chloro and methylthioatrazine herbicides as well as monodealkylated chlorotriazines from urine samples	Mendaš et al. 2001

1. There are multiple methods for extracting, cleaning up, and otherwise preparing samples for effective analysis of triazine herbicides.
2. Although the most common methods in use are effective, some are more suitable than others, because of cost, availability of laboratory equipment, or the required limit of detection (LOD).
3. The most popular technique for securing clean extracts is SPE. The popularity of this technique results from its advantages: requires small solvent volumes, no emulsion formation, different types of sorbents available, and adequate for many analytes (Namieśnik et al. 2000).

### **3 Analytical Methods for Determining Triazines in Environmental Samples**

The most popular techniques for atrazine and simazine determination in soil, vegetable, fruit, animal tissue, milk, egg, and urine samples are high-pressure liquid chromatography (HPLC) and gas chromatography (GC). Capillary electrophoresis (CE) is an alternative to the conventional HPLC and GC techniques and is gaining importance.

Immunoassays (especially through the use of kits) are also becoming available and enable triazine analysis under field conditions. Older techniques that are still sometimes used for analysis of triazines include thin-layer chromatography (TLC) and spectrophotometry; but as the requirements for achieving low detection limits of analytes increase, these techniques are diminishing in importance.

#### ***3.1 High-Pressure Liquid Chromatography***

HPLC is commonly and broadly used for analysis of xenobiotic contaminations in the environment. A wide spectrum of detectors is available for HPLC instrumentation, and innovative sample preparation techniques enable analysis of xenobiotics at low concentrations in complex environmental matrices.

The conditions under which atrazine and simazine are commonly analyzed by HPLC are summarized in Table 8.

#### ***3.2 Gas Chromatography***

Gas chromatography preceded HPLC, but came into use later than did spectrophotometry and TLC. Today, among the most significant and popular techniques is GC coupled to mass spectrometry (MS). This combination allows higher certainty of the identity of low levels of residues. Nevertheless, GC can only be applied for the

**Table 8** Conditions under which atrazine and simazine are commonly analyzed using HPLC

Analyte	Sample type	Column	Mobile phase	Detector	LOD	Reference
Atrazine, simazine	Ground water	C18	Methanol	UV, $\lambda = 220$ nm	40 ng L <sup>-1</sup> (atrazine) 20 ng L <sup>-1</sup> (simazine)	Abgekodo et al. 1996
Atrazine and metabolites, simazine	Ground and surface water	C18, 250 × 4.0 mm, 5 $\mu$ m	Acetonitrile – 20 mM phosphate buffer, pH 7 (gradient elution)	UV, $\lambda = 25, 240, 260$ nm	0.02 $\mu$ g L <sup>-1</sup>	Carabias-Martinez et al. 2002
Atrazine, simazine	Tap water	C18, 250 × 4.0 mm, 5 $\mu$ m	Methanol-water (60:40 v/v)	LC-MS	0.1 $\mu$ g L <sup>-1</sup>	Hogenboom et al. 1997, 1999; Hogendoorn and van Zoonen 2000; Borba da Chuna et al. 2004
Simazine, propazine, hexazinone, metoxuron, bromacil	Surface water, soil	C18, 250 × 4 mm, 7 $\mu$ m; C8 250 × 4 mm, 7 $\mu$ m	Methanol-water (3:1; 1:1, 2:1 v/v)	DAD	0.06–0.3 $\mu$ g L <sup>-1</sup> (water), 0.24–1.4 $\mu$ g kg <sup>-1</sup> (soil)	Baranowska and Pieszko 2000b
Atrazine	Ground and surface water	C18, 125 × 4.6 mm, 5 $\mu$ m	Methanol-water (60:40 v/v)	DAD	6 ng ml <sup>-1</sup>	Kumazawa and Suzuki 2000
Atrazine, simazine	Ground water	C18, 250 × 4.6 mm, 5 $\mu$ m	Methanol-water (73:27, v/v), pH 6	FL, $\lambda_{\text{ex}} = 312$ nm, $\lambda_{\text{em}} = 420$ nm	1.2 ng g <sup>-1</sup> (atrazine), 1.1 ng g <sup>-1</sup> (simazine)	Gong and Ye 1998
Simazine	Water, apple juice	C18, 150 × 0.18 mm, 3 $\mu$ m	Acetonitrile-water (gradient elution)	UV, $\lambda = 220$ nm	0.15 $\mu$ g L <sup>-1</sup>	Liu and Lee 1998
Atrazine	Ground water	C18	Methanol-water (65:35 v/v)	UV, $\lambda = 220$ nm	0.05 $\mu$ g kg <sup>-1</sup>	Ribeiro et al. 2005
Atrazine, simazine	Soil	C18, 200 × 2.1 mm, 4 $\mu$ m	Acetonitrile-water (gradient elution)	DAD	0.5–2.0 $\mu$ g L <sup>-1</sup>	Schutz et al. 1994; Ying et al. 2005
		C18, 100 × 5.0 mm, 4 $\mu$ m	Acetonitrile-water (30:70 v/v)	UV, $\lambda = 220$ nm	n.r.	Singh et al. 2004
		C18	Methanol-water	DAD, $\lambda = 254$ nm	1.5 $\mu$ g kg <sup>-1</sup>	Smith 2002
		C18	Methanol-water	FL	n.r.	Barriuso et al. 1996

(continued)

Table 8 (continued)

Analyte	Sample type	Column	Mobile phase	Detector	LOD	Reference
Atrazine, simazine, metribuzin	Beet, celery, cereals	C18, 125 × 4.0 mm, 5 µm	Acetonitril –20 mM phosphate buffer, pH 6.7, gradient elution	UV, λ = 220 nm	10 ng g <sup>-1</sup>	Fahrenhorst et al. 2000; Prosen and Zupancic-Krajc 2005
Atrazine	Carrots, potatoes	C18	Methanol–ammonium formate 10 mM	MS–MS	0.5–2.0 µg kg <sup>-1</sup>	Nú ez et al. 2005
Atrazine	Oranges, maize	C18	Methanol–water (70:30 v/v)	UV, λ = 220, 230 nm	0.015–0.300 ppm	Wittmann and Hoek 1993
Simazine	Blue berries, fruit juices	C18	Methano–acetic buffer (gradient elution)	UV, λ = 230 nm	0.08 ppm (blueberry), 20 µg L <sup>-1</sup> (juices)	Tadeo et al. 2000
Atrazine, simazine	Soil, plants, animal tissues, milk, eggs	C18, 250 × 4.6 mm, 5 µm	Methanol–water (1:1 v/v)	DAD, λ = 222 nm	0.06–0.80 ng g <sup>-1</sup>	Baranowska et al. 2005, 2006, 2008; Price et al. 2006
	Vegetables, roots and leaves, fruits, cereals				0.5–4.3 ng g <sup>-1</sup>	
	Milk				0.3 ng g <sup>-1</sup>	Baranowska et al. 2006
	Eggs				3.2–3.9 ng g <sup>-1</sup>	Baranowska et al. 2005;
	Pig, duck, rabbit meat and fat				0.3–0.6 ng g <sup>-1</sup>	Baranowska et al. 2005; Barchańska 2007
						Baranowska et al. 2006; Barchańska 2007



analysis of volatile and reasonably stable substances, or ones that can be rendered volatile through derivatization.

The most often used GC detectors are FID (Flame Ionization Detection), NPD (Nitrogen-Phosphorus Detection), MS (Mass Spectrometry), and FPD (Photometric Detection). Conditions under which triazines are determined by GC are presented in Table 9.

### 3.3 *Thin-Layer Chromatography*

Atrazine in water-dichloromethane extracts, from soil samples, was determined using the following TLC media: RP-18 F<sub>254</sub>. The mobile phases were either methanol-water (8:26 v/v) or chloroform-methanol-formic acid (80:19:1 v/v/v). Spots were exposed to UV light (254 nm) (Kördel et al. 1995; Babić et al. 1998). Vegetable extracts were developed in a mixture of chloroform-methanol (9:1 v/v) (De Prado et al. 2000).

The different conditions used for TLC analysis of ametryn, atrazine, propazine, and simazine extracts from drinking water samples were as follows: stationary phase: silica gel 60 F254, mobile phase: *n*-heptane-ethyl acetate (1:1 v/v); stationary phase: C18 F254, mobile phase: acetonitrile-water (7:3 v/v); stationary phase: Diol F254, mobile phase: acetone-water-tetrahydrofuran (4:6:1 v/v/v). According to the authors, the last two sets of chromatographic conditions enable fast analysis with a detection limit of 0.1 µg L<sup>-1</sup> (Hamada and Wintersteiger 2002).

TLC and 2D-TLC (two-dimensional TLC) were used for simazine, propazine, and phenylurea herbicide (applied to 14 compounds) determination. The stationary phases used were silica gel 60 F254 and RP-18; the mobile phases were methanol-water (60:40 v/v) followed by tetrahydrofuran-*n*-heptane (20:80 v/v). Spots were visualized using iodine vapors or UV light (254 nm) (Tuzmiński and Soczewiński 2002).

### 3.4 *Capillary Electrophoresis*

Capillary electrophoresis (CE) facilitates rapid separation of many types of chemical compounds. This technique is sensitive and has the advantage of requiring only small sample volumes. It is growing in importance as a method for analysis of triazines in environmental samples (Carabias-Martinez et al. 2000, 2006; Turiel et al. 2000; Picó et al. 2003). CE was used for determination of atrazine, simazine, prometryn, and propazine and their metabolites in ground and surface water samples (Carabias-Martinez et al. 2000, 2006); chloric acid (7.5·10<sup>-3</sup> mol L<sup>-1</sup>) in methanol-acetonitrile (50:50 v/v and 30:70 v/v) were used as the electrolyte, and respectively retained a potential of 22 kV and 10 kV. In contrast, another author applied micellar electrokinetic chromatography, and used a 10 mM solution of potassium phosphate (pH 9.0, 30 mM SDS) with addition of 8% methanol as the electrolyte (Menzinger et al. 2000).

**Table 9** Conditions under which atrazine and simazine are analyzed using GC

Analyte	Sample type	Column	Detector	LOD	Reference
Atrazine, simazine, prometryn, metribuzin	Surface and ground water	DB-1, CP-SIL 13 CB	ECD, NPD	0.06 $\mu\text{g L}^{-1}$	Dean et al. 1996; Vassilakis et al. 1998
Atrazine, simazine	Water, soil	SPB-5	NPD	2 $\mu\text{g kg}^{-1}$ (soil), 0.02 $\mu\text{g L}^{-1}$	Funari et al. 1998
Atrazine, simazine	Water, sediments	SPB-5	NPD	50 $\text{ng L}^{-1}$ (water), 2 $\text{ng g}^{-1}$ (sediments)	Meakins et al. 1996
Atrazine	Soil	DB-5	NPD	1.5 $\mu\text{g kg}^{-1}$	Sabik et al. 1995
Simazine	Vegetables, cereals	OV-101	NPD	0.1 $\mu\text{g kg}^{-1}$ (vegetables), 0.02 ppm (cereals)	Tadeo et al. 2000
Atrazine, simazine, metribuzin	Vegetables, cereals, apples	OV-225, DB-17	NPD		
Atrazine	Maize				
Atrazine, simazine	Grass	SPB-5	MS	2 $\mu\text{g kg}^{-1}$ (atrazine), 6 $\mu\text{g kg}^{-1}$ (simazine)	Johnson et al. 1997
Atrazine, simazine	Vegetation	SDB	NPD	n.r.	Sadeghi and Isensee 2001
atrazine, simazine	Olive oil	Cyanopropylphenyl	NPD	0.005 $\text{mg kg}^{-1}$ (LOQ)	Lentza-Risos et al. 2001
Atrazine, simazine, prometryn, propazine	Human blood serum, soil	DB-5	MS	5 $\mu\text{g L}^{-1}$ ; 2-4 $\mu\text{g kg}^{-1}$ (depending on the compound)	Lacassie et al. 2001; Ribeiro et al. 2005
Atrazine, simazine, prometryn, propazine	Human blood serum, urine	DB-1	FID, NPD	0.2-1.4 $\mu\text{g mL}^{-1}$ (FID) 20-60 $\mu\text{g mL}^{-1}$ (NPD), depending on the compound	Kumazawa and Suzuki 2000
Atrazine, simazine, and their metabolites	Human urine	DB-17	NPD	5.6-18.0 $\mu\text{g mL}^{-1}$ depending on the compound	Mendaš et al. 2001
		SPB-5	ECD, NPD	10 $\text{ng mL}^{-1}$	

When triazines were analyzed by means of capillary zone electrophoresis, the electrolyte used was a 100 mM sodium acetate solution (pH 4.6) with addition of 4% methanol. Detection was carried out at  $\lambda = 210$  nm and  $\lambda = 214$  nm by means of diode array detection. The detection limits were 0.02–0.32  $\mu\text{g L}^{-1}$ , depending on matrix.

### 3.5 Spectrophotometry

Spectrophotometry is an inexpensive and widely available technique that can be used to analyze triazines.

Atrazine residues in soil, vegetables, seeds, and water were determined by spectrophotometry (Kesari and Gupta 1998; Ojeda and Rojas 2004). A derivatization step was required to foment spectrophotometric detection of target residues. Ojedas and Rojas (2004) used *p*-aminoacetophenone as the derivitizing agent. The influence of this agent on the atrazine determination of other pesticides and ions was investigated. Absorbance was measured at  $\lambda = 470$  nm and the analytical level of detection was in the range of 0.16–1.6 ppm.

Derivative spectrophotometry (zero-crossing technique) was applied to the determination of selected phenols and herbicides in a two-component mixture. Methyl- and chloro-phenols, and simazine, propazine, hexazinone, bromacil, and metoxuron were examined. The recoveries were between 97–110% (Baranowska and Pieszko 2000a, b).

### 3.6 Immunoassay

Immunoassay, as a technique to identify xenobiotic residues, was reported by Yalow and Berson (1960). The use of such assays have now been researched for years, have been refined, and are increasingly available as an alternative to conventional chemical residue analysis. The immunoenzymatic and immunochromatographic methods that are increasingly being used are selective, sensitive, and generally easy to use (Pacáková et al. 1996).

Enzyme-linked immunosorbent assay (ELISA) has been applied for the analysis of triazines in several sample types: soil (Xiong et al. 1998), fruit extracts (Franek et al. 1995; Delaunay et al. 2000; Ralph 2000), surface and ground water (Van Emon and Lopez-Avila 1992; Gascón et al. 1997; Mallat et al. 2001; Abuknesha and Griffith 2004), milk (Franek et al. 1995), mouse kidney and liver (LOD 100 ng  $\text{L}^{-1}$ ; Thrumann et al. 1990; Scutaru et al. 1998). ELISA provided low detection limits, and no matrix interferences; moreover, ELISA is fast and may be conducted under field conditions.

Atrazine and simazine were determined in environmental samples by means of immunosensors, the detection limits of which were 0.025–0.1  $\mu\text{g L}^{-1}$  (Sparr Eskilsson and Björklund 2000; Mallat et al. 2001).

Atrazine and simazine were determined by Price et al. (2006) and Baranowska et al. (2008) in trophic chain samples. The conditions used for these ELISA assays enabled the determination of atrazine and simazine in soil samples, and vegetation and animal tissues, after extraction with chloroform, and dilution of the residue extract solution. No additional clean-up was necessary. In comparison with HPLC, ELISA assays enable faster analysis with a lower limit of detection. Moreover, the time necessary to analyze triazine extracts with ELISA (156 min) is much shorter than for GC (236 hr), for example. In addition, analysis of water samples using ELISA requires essentially no preparation (McLaughlin et al. 2008).

There are commercially available immunoassay kits produced or sold by several enterprises: Ohmicron, Riedel-deHaen, J.T.Baker, Avena GmbH, Millipore, Transia, Abraxis. These kits allow triazine herbicide determination in environmental samples with detection limits that range between 0.005 and 5.0  $\mu\text{g L}^{-1}$  (Mouvet et al. 1995; Gascón et al. 1997; Menleuberg et al. 1999; Ribeiro et al. 2005).

The choice of analytical method to analyze for environmental pollutants depends on the chemical structure and volatility of the agents under analysis. HPLC is usually used when pesticides are not suitable candidates for GC analysis. Advances in HPLC detection and column material technology have accentuated the importance of this method for pesticide residue analysis. Use of immunosorbents and molecular imprinted polymers has broadened and strengthened the spectrum of applications for HPLC pesticide analysis. Moreover, the creation of immunoassays has enabled the analysis of pesticides in environmental samples without the need for time-consuming sample pretreatment. Immunoassay techniques have also provided, for the first time, a realistic and relatively simple tool for determining environmental residues under field conditions.

## 4 Summary

There is an ongoing need to monitor soil and trophic chain samples for residues of triazine herbicides, particularly atrazine and simazine, because these herbicides are among the most used members of their class, are toxic, can be persistent, and are widely distributed in the environment. The main purpose of this review is to provide an overview of principle techniques and approaches used in analyzing atrazine, simazine, and other triazine herbicide residues in environmental matrices. The methods covered generally provide low detection limits, acceptable levels of matrix interferences, and are relatively fast and inexpensive.

Atrazine and simazine are popular herbicides used to control a variety of broad leaf and grassy weeds in agriculture and on industrial sites. Because they are widely and frequently used, the environmental contamination of these compounds is considerable. Atrazine, simazine, and other triazines have the ability to translocate in

ecosystems. When this occurs, it is often necessary to monitor their residue content in soils, vegetation, biota, and water. There is a vast literature available that addresses the extraction and clean-up of soil, vegetation, animal tissue, and animal fluid samples; unfortunately, few of these publications compare the effectiveness of results obtained on similar matrices. In this review we endeavor to review and provide comparative information on methods dedicated to determining residues of atrazine, simazine, and other triazines in several environmental matrices: soil, plants, animal tissues, and water.

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