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Impacts of Pesticides on Arthropod Biological Control Agents

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ABSTRACT

Information about the side-effects of pesticides on biological control agents is an essential requirement of integrated pest management (IPM). Different methods to test the effects of pesticides on natural enemies have been used and new methods are being developed. In the past, evaluations were mostly based on individual level (lethal or sublethal) endpoints. Differences in the used methods and the measured endpoints make it difficult to compare the results. There is increasing emphasis on using standard methods to combine the lethal and sublethal effects to a total effect. Especially, population-level effects or demographic toxicology has been concluded as a better measure because of its ecological relevance and is the current centre of attention. Very recently, molecular and biochemical methods, primarily, have been developed for detecting potential damage to populations at early stages. But these types of responses (i.e. biomarkers) to toxic stress are only demographically relevant if the response can be linked to effects at higher organism levels. We describe the methods used to study the effects of pesticides on beneficial arthropods and the current status of the evaluation of side-effects. We also provide new suggestions. In addition to methodological discussion, in the last part we presented a table containing summary database on the effect of key classes of commonly used pesticides on various natural enemies. This data may be helpful for researchers or IPM users.

Keywords: biomarker, demographic toxicology, natural enemies, side-effects, total effect

Abbreviations: **BART**, beneficial arthropod regulatory testing; ***Bt***, *Bacillus thuringiensis*; **CEA**, cellular energy allocation; **DT**, doubling time; **ECx**, the concentration causing an effect of x per cent; **EPPO**, European and Mediterranean Plant Protection Organization; **ETS**, electron transport system; **EU**, European Union; **FAO**, Food and Agriculture Organization; **ICES**, International Council for the Exploration of the Sea; **IGRs**, insect growth regulators; **IOBC**, International Organization for Biological Control of Noxious Animals and Plants; **IOBC/WPRS**, International Organization for Biological Control of Noxious Animals and Plants/West Palearctic Regional Section; **IPM**, integrated pest management; **LC50**, median lethal concentration; **LD50**, median lethal dose; **LTREs**, life table response experiments; **OECD**, Organization for Economic Co-Operation Development; **NEC**, no effect concentration; **NOEC**, no observed effect concentration; **NOEL**, no observed effect level; **PBO**, piperonyle butoxide

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INTRODUCTION

The significance of biological control (BC) agents for the management of agricultural pests has been increasingly realized during the last three decades; however BC is not a panacea for all pest problems. The integration of biological and chemical control agents is more effective for the management of insect pests. In fact, pesticides and BC agents are two important com-ponents of integrated pest management (IPM). But the use of pesticides must be compatible with the other agents of pest management. Most contact insecticides from different chemical classes are broad spectrum and so affect both prey and predator. A few physiologically selective pesticides from each class are available that may be used in IPM. A physiologically selective pesticide is one that is toxic to some pests, but has little or no effect on other similar species. Ecological selectivity, on the other hand, can be accomplished by manipulation of the pesticide formulation, timing of application, method of application, spatial distribution of treatment, and other means (Croft 1990)

Pesticides have direct and indirect effects on beneficial arthropods. Studies on the direct effect of pesticides are done by measurement of toxicity to beneficial arthropods and

Received: 15 May, 2008. Accepted: 17 September, 2008.

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determination of the median lethal dose (LD50) or lethal concentration (LC50). In the past, comparison of LD50 or LC50 values of insecticides to both BC agents and pests was vastly used to estimate selectivity. Based on LD50 beneficial arthropods are even more susceptible to insecticides than insect pests. Because these evaluations focus on a single life stage and generally for a short duration of time (often 1-4 days), the results of these bioassays do not accurately assess the total effects of a pesticide on an exposed population (Stark and Banken 2000). Therefore, in order to determine the total effect of a pesticide, one has to take into account the indirect or sublethal effects of pesticides, too. Sublethal doses of pesticides can affect the physiology and behavior of the BC agents.

Different methods to test sublethal effects on natural enemies are being developed. Effects such as altered behavior, reduced reproduction, and reduced longevity of non-target organisms are the conspicuous consequences of sublethal doses. These effects may be seen in different developmental stages of non-target arthropods. Like mortality, sublethal effects can severely reduce the performance of BC agents (Elzen *et al.* 1989; Roger *et al.* 1995). By far, demography or life table response experiments (LTREs) have been suggested as a desirable means to evaluate the total effect of pesticides on natural enemies. LTREs take into account all effects that a toxicant might have at the levels of organization higher than the individual (Stark *et al.* 1997, 1998, 2004). The advantage of this ap-proach is that a total measure of the effect is determined that incorporates lethal and sublethal effects into one end-point, the intrinsic rate of natural increase (Stark *et al.* 1998; Stark and Banks 2000, 2004). Most traditional pesti-cides are broad-spectrum organic compounds that wipe out populations of beneficial as well as different pests. That is because almost all four groups of traditional insecticides targeted the nervous system, which is biochemically similar in beneficial and pests (van Emden 1996; Rechcigl and Rechcigl 2000).

The literature on natural enemy/pesticide research has grown rapidly since the mid 1970s. Excellent reviews and books have been published during the last 30 years (Croft and Brown 1975; Smith and Stratton 1986; Croft 1990; Stark and Banks 2003; Desneux *et al*. 2007). Generally most traditional insecticide classes such as organophosphates, carbamates and synthetic pyrethroids are highly toxic to beneficial arthropods. Therefore it is hard to find a true selective compound among traditional toxic insecticides. In many crops the most widely used insecticide class is now the organophosphates. Some organophosphates are somewhat selective. The mite predators (*Neoseiulus fallacies*, German) of orchard spider mites have acquired their own resistance to organophosphates. On the other hand, partial selectivity can be attained in application when take advantage of formulations. Systemic organophosphate insecticides such as demeton-s-methyl, dimethoate and acephate are selective for natural enemies of aphids and mites. The carbamate insecticide pirimicarb is toxic to aphids and Diptera, yet not to other insects at equivalent doses (van Emden 1996). The organochlorine endosulfan is selective for Hymeonoptera which include valuable BC agents. Some newer classes of insecticides such as pyrethroids are extremely toxic to insects. However, even in this class of insecticide a single compound such as fluvalinate is selective for honeybees (Hill 1985; Walter *et al*. 1988).

The newly marketed insecticides with a novel mode of action are less toxic to beneficial arthropods. Azadirachtin, indoxacarb, spinosad and pymetrozine are extremely toxic to target pests, while significantly less toxic to natural enemies (Boyd and Boethel 1998; Babul Hossain and Poehling 2006). The selectivity and low toxicity make them convenient for utility in Integrated Pest Management (IPM). The type of formulation affects their toxicity to non-target organism including natural enemies. Some formulations are less toxic to beneficial insects and mites. For example granule and soil-applied formulations are less toxic than spray simply because they do not leave a residue on the leaf surface. Systemic pesticides injected or applied to soil pose minimal hazard to beneficial arthropods. Wettable powders and microencapsulated formulations are the most toxic.

The aim of this paper is to review the development of methods for measurement and interpretation of the side-effects of pesticides on BC agents. Aspect of this topic such as interpretation of standardized side-effect testing, lethal, sublethal, and multiple endpoints are discussed. The second part of the review is concerned with the impact of individual pesticide classes on selected natural enemies. In this part we summarize the results of some toxicity testing using different methods in a table.

METHODS OF EVALUATING SIDE-EFFECTS OF PESTICIDES

Scientific methods are needed to assess the risk of pesticides on natural enemies and apply as pre-registration tools as well as determine the compatibility with IPM after registration (Stark *et al.* 1995; Jepson and Croft 1998).

Various types of pesticide effects on arthropod biocontrol agents have been studied and reviewed several times (Croft 1990; Stark and Banks 2003; Desneux *et al.* 2007).

Different methods and endpoints used to study the effects of pesticides and the current statuses as well as the new suggestions are discussed below.

Pre-registration research

Data on physiology and toxicology of arthropod natural enemies has been mainly extrapolated from phytophagous species and there is not enough knowledge in this respect. On the other hand effective methods to study the side-effects of pesticides at earlier stages of their development and early detection of potential hazards have not been developed.

These problems are considered as the reasons for limited research in the pesticide development process (Jepson and Croft 1998). However, specific guidelines have been developed in order to test side-effects for registration of plant protection products. The Organization for Economic Co-Operation Development (OECD) with 30 member countries worldwide has developed guidelines for registration requirements of various products (OECD, 2002, 2003). In the European Union (EU) it is currently conducted according to the Council Directive of 91/414/EEC. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) is followed in the United States (Candolfi *et al.* 2000; Desneux *et al.* 2007).

Post-registration research

Individual-level effects

Toxic substances can cause changes at all levels of biological organization from molecular to community (Hyne and Maher 2003). Traditional methods based on individual level endpoints are dominant in the literature. Acute and chronic toxicity tests are the two focal ways to study the impacts of pesticides on individuals.

Acute toxicity tests

LD50 or LC50, have been used mostly to measure the toxic effects of pesticides on beneficial arthropods (Desneux 2007).

LD50 is an estimate of the dose that causes 50% mortality of a group of individuals under test. A sigmoid curve is fitted to the number of survivors as a function of the dose of toxicant. This curve is usually the log-logistic or log-probit curve (Finney 1971). In some cases the exact dose originally given to the insect (e.g. larval stage of aquatic insects) cannot be determined but the concentration of the insecticide in the peripheral media can, so that the LC50 is used (Matsumura 1985). LD50 have also been used to estimate the selectivity ratios (LD50 of the beneficial/pest arthro-

pods) (Croft 1990).

Acute toxicity tests use single endpoint (mortality) and are performed during short duration (1 to 4 days in many cases) (Walthall and Stark 1997). This kind of studies has been used on both parasitoids and predators (Rosenheim and Hoy 1988; Mizell and Sconyers 1992; Hamilton and Lashomb 1997; Desneux *et al.* 2003).

Chronic toxicity tests

Besides lethal effects pesticides may cause some important sublethal effects on individuals that survive the toxicant exposure. Short-term acute toxicity tests usually ignore this kind of effects (Laskowski 2001). Some examples of endpoints of interest in chronic studies are fecundity, body size, development rate, behavior, sex ratio, and longevity (Desneux 2007).

For risk assessment purposes there is a need to determine low or no toxic effect levels (van Leeuwen and Hermens 1995; Koojman *et al.* 1996; de Bruijn and Hof 1997; van der Hoeven 1997). Several measures have been proposed to use as estimates for these concentrations.

NOEC (no observed effect concentration), NOEL (no observed effect level), ECx (the concentration causing an effect of x per cent), and NEC (no effect concentration) can be applied to various endpoints of sublethal effects. The negative and positive aspects of these measures have been previously discussed (Moore and Caux 1997; van der Hoeven 1997; Crane and Newman 2000; van der Hoeven 2004).

Total effects

One of the limitations of traditional methods is that sublethal and lethal effects are not combined and thus the total effect of a pesticide is not determined (Stark and Wennergren 1995). Several efforts have been made and methods have been introduced to solve this problem. These efforts and methods are described below.

IOBC methods

These methods were developed by the 'pesticides and beneficial organisms' working group of the International Organization for Biological Control (IOBC). The working group was founded in 1974 and its major aim was to encourage the development of standard methods for testing the side-effects of pesticides on natural enemies to support the IPM. A further aim was therefore to test the side-effects of commonly used pesticides on the most important natural enemies (Hassan 1998a).

The group established cooperation with other international organizations such as the Beneficial Arthropod Regulatory Testing (BART), the European and Mediterranean Plant Protection Organization (EPPO), the European Union (EU), and the Food and Agriculture Organization (FAO) (Hassan 1998b).

The IOBC method has been designed to evaluate the acute residual toxicity as well as sublethal effects of pesticides on reproductive performance (Vogt *et al*. 2000). The mean mortality (M) and average fecundity (R) are measured and then the total effects of the pesticides (E%) are calculated by the formula proposed by Overmeer and Van Zon (1982):

$$E\% = 100 - (100 - M) \times R \times 100.$$

There are several works in which this formula have been used to take into account both lethal and sublethal effects on the reproductive performance (Oomen *et al*. 1991; Blümel *et al*. 2000; Van de Veire *et al*. 2002; Kavousi and Talebi 2003; Rezaei *et al*. 2007; Sáenz-de-Cabezón Irigaray *et al*. 2007).

Based on the total effects the pesticides are classified using IOBC evaluation categories (Sterk *et al.* 1999).

Recognizing that no single test method would provide sufficient information to assess the side-effects of pesticides on a beneficial organism, a combination of tests is recommended. The IOBC suggests a sequential scheme in which pesticides are first tested in the laboratory. If no meaningful effect is observed, they are considered compatible for use in IPM programs. In the case of a meaningful adverse effect in the laboratory, tests are further performed under semi-field conditions. If significant effects are still observed in this tier a more complex field study is considered to assess the impact of the pesticide under realistic field conditions. Compounds with no significant adverse effects in the semi-field and field experiments are recommended for use in IPM (Dohmen 1998).

Laboratory experiments are conducted under 'worst case' conditions which aims to ensure a maximum exposure of the organisms to the test substance. In the semi-field tests no extreme exposure, as in the laboratory tests, is applied; however, a realistic worst case with respect to exposure is simulated. In the last stage, extensive field tests may be employed.

Oomen *et al.* (1991) reported the side-effects of 100 pesticides on the predatory mite *Phytoseiulus persimilis* using a combination of laboratory, semi-field and field tests. Van de Veire *et al*. (2002) described laboratory to field sequential testing scheme for testing side effects of pesticides on anthocorid bugs using *Orius laevigatus* as the test species.

The 'pesticides and beneficial organisms' working group of the IOBC develops standard methods based on laboratory to field tests to evaluate the side-effects of pesticides on important beneficial organisms. Joint pesticide testing programs by members of the working group have been organized every two years since 1977. Since 1980, the results of seven joint pesticide testing programs carried out by the IOBC/WPRS-Working Group 'Pesticides and Beneficial Organisms' have been published. Within these seven programs more than 120 pesticides have been tested on various beneficial organisms including arthropod natural enemies using laboratory, semi-field and field methods (Sterk *et al.* 1999). Stark *et al.* (2007) have criticized IOBC approach and argued that the ecological relevance of IOBC methods is questionable.

Life table studies

To better estimate the side-effects of pesticides there is an increasing attention and awareness to use more realistic endpoints. Demography or LTREs have been suggested as the best way to combine lethal and sublethal effects and use to estimate the total effects of pesticides (Daniels and Allan 1981; Bechmann 1994; Stark and Wennergren 1995; Stark *et al.* 1998; Forbs and Calow 1999; Stark and Banks 2003; Robertson *et al.* 2007; Stark *et al.* 2007). Experiments in which life tables, or more generally a set of vital rates, are the dependent variable are called LTREs. Demographic toxicological analysis incorporates survivorship and reproduction of test organisms into one endpoint (i.e. population growth rate) (Caswell 2000).

Population growth rate is expressed as intrinsic rate of increase (r) or finite rate of increase ($\lambda = e^r$). To calculate the population growth rate, life tables are constructed using a cohort of the test organism exposed to the toxicant. The probability that a new individual is alive at age x (l_x) and the number of female offspring produced by a female with attributed x (m_x) are recorded. From these two functions, the Maltusian parameter (population growth rate), r, is calculated using Euler's equation:

$$\int_{x=0}^{\infty} l_x m_x e^{-rx} dx = 1$$

Positive values of r indicate an exponential population increase, the value equal to zero indicates the stable state of a population, and r values less than zero indicate that the population is declining exponentially and heading to extinction (Carey 1993).

The rate of increase of a population can also be generated using matrix algebra. In this method the age distribution of the population at time t (N_t), considered as a column

vector (n_t) is multiplied by a transition matrix referred to as Leslie's matrix or population projection matrix (L) to get the age distribution at time t+1 (N_{t+1}):

$$N_{t+1} = L \times N_t$$

$$\begin{bmatrix} n_{0,t+1} \\ n_{1,t+1} \\ n_{2,t+1} \\ n_{3,t+1} \end{bmatrix} = \begin{bmatrix} f_0 & f_1 & f_2 & f_3 \\ s_0 & 0 & 0 & 0 \\ 0 & s_1 & 0 & 0 \\ 0 & 0 & s_2 & 0 \end{bmatrix} \begin{bmatrix} n_{0,t} \\ n_{1,t} \\ n_{2,t} \\ n_{3,t} \end{bmatrix}$$

f_x = Age-specific fecundity, s_x = Age-specific survival rate, n_x = Number of individuals of age x.

Repeated iterations of the multiplication of the population vector and the transition matrix result in a stable population vector (stable age distribution). At this condition the population is multiplied by a constant factor per time interval. This factor, the dominant eigenvalue of the projection matrix, is the finite rate of increase (λ).

An alternative population growth rate, the instantaneous rate of increase (r_i) has been introduced that simplifies the gathering of population-level data. It reflects the actual growth of a population and is calculated by the following equation:

$$r_i = \ln(N_f / N_0) / \Delta T$$

where N_f is the final number of animals, N_0 is the initial number of animals and ΔT is the change in time (Walthall and Stark 1997).

To date most life table data have been collected using only the female individuals. Chi (1988, 2005) introduced new method to conduct life tables using both females and males (age-stage, two-sex life table analysis) and developed computer software to calculate the life table parameters.

All above mentioned population growth rates measure the numerical aspects of population. A new population level index has been proposed (van Straalen and Kammenga 1998) that measures the qualitative aspects of the population. This index named 'intrinsic biomass turnover' measures the productivity of the population (rate of which biomass is produced, relative to the biomass present).

Advantages of measuring population level effects

Several authors have emphasized the priority of population level effects in comparison to various individual level endpoints. The greatest value of the use of population parameters lies in their ecological relevance, and the possibility of summarizing a variety of possible effects in the course of a life-cycle by a single measure (Daniels and Allan 1981; Allan and Daniels 1982; Stark *et al.* 1997; Stark *et al.* 1998; Kammenga and Laskowski 2000; Stark and Banks 2003).

Forbs and Calow (1999) reviewed the literature and concluded that population growth rate is a better measure of response to toxicants than the individual-level endpoints. Their reasoning was that it integrates potentially complex interactions among life-history traits and provides a more relevant measure of ecological impact. They also mentioned that r makes it possible to evaluate the conflicting effects of toxicants on survival and reproduction.

For example, it has been demonstrated that aphids could maintain high population growth rates after exposure to LC60 (Walthall and Stark 1997). On the other hand Bechmann (1994) showed that in some cases the demographic parameters may be affected by sublethal concentrations (32% of LC50). It has been demonstrated that in such cases the extinction of population may occurs by sublethal concentrations. Thus, short-term acute toxicity tests may overestimate or underestimate the effects of different pesticides. This shows the necessity of conducting long-term assays (e.g. life table studies) to find the realistic results on impacts of pesticides. Another advantage of studying the effects of toxic substances on populations rather than individuals can be seen in case of compounds like insect growth regulators (IGRs) which have slow action. This kind of products cannot be adequately evaluated using short-term laboratory tests based on individual level endpoints (Stark *et al.* 1998; Sáenz-de-Cabezón *et al.* 2006). Stark *et al.* (1997, 1998) showed that different species may show similar acute susceptibility to certain pesticides while their responses at population level are very different. Besides, Kim *et al.* (2006) showed that different pesticides may cause comparable acute toxicity but completely different total effect on a known species.

Biomarkers

Toxic substances can cause changes at all levels of biological organization from molecular to community (Hyne and Maher 2003). Processes at one level take their mechanisms from the level below and find their consequences at the level above. The ecological relevance increases from the sub-cellular to the ecosystem level (De Coen 1999). As mentioned above, several authors have suggested that studying population level effects of toxicants by use of demographic toxicological analysis is the best approach and provides a more relevant measure of ecological impact. In demographic toxicology both lethal and sublethal effects are combined into one integrative parameter, the intrinsic rate of increase (r_m) (Bechmann 1994; Stark and Wennergren 1995; Forbs and Calow 1999; Stark and Banks 2003). The major disadvantage to the use of demographic toxicology is that development of life table data is expensive and time consuming (Forbs and Calow 1999; Stark and Banks 2003). In addition, an understanding of the population level effects of a toxicant without understanding of the damage that a toxicant causes at biochemical level and knowledge of how it is causing these effects is not enough (Stark and Banks 2000). Kammenga and Laskowski (2000) refer to the relationship between toxicant-induced biochemical or cellular alternations (i.e. biomarker responses) and subsequent demographic changes as a pressing problem in ecotoxicology. Biomarkers are useful as markers for both exposure and effect in organisms (Pretti and Cognetti-Varriale 2001) and could be used in monitoring for effects before they reach the population or community levels (Lagadic *et al.* 1994). Invertebrate biomarkers and their use in monitoring of ecosystem quality have been reviewed and the lack of knowledge on the linkages between biomarker and population level responses has been highlighted. The potential use of biomarkers related to energy metabolism for predicting effects of stressors on population structure and dynamics have been mentioned by several authors (Lagadic *et al.* 1994; De Coen and Janssen 1997; Lagadic 1999; De Coen *et al.* 2000; Verslycke and Janssen 2002; De Coen and Janssen 2003). Bio-energetic or physiological energetic, in general, offer the advantage to provide information on key processes in the organism's energy acquisition and expenditure, possibly also elucidating the mode of action of the toxicant. Moreover, changes in the energy metabolism, in general, will ultimately influence the future life characteristics of an organism (De Coen *et al.* 2000). The response of biomarkers to toxic stress is only demographically relevant if the response can be linked to effects at higher organism levels, such as individual (life-cycle traits) or population level (Kammenga and Laskowski 2000).

Recently a biomarker has been developed based on "metabolic cost" hypothesis called Cellular Energy Allocation (CEA). This methodology could provide an integrative quantification of the organism's energy budget based on a biochemical comparison of the organism's energy consumption and the energy reserves available for metabolism (De Coen *et al.* 2000). Energy consumption is determined by measuring the electron transport activity (ETS activity) which is a biochemical measure of the potential metabolic activity and is nearly universal in all organisms (Packard *et al.* 1971). The energy reserves are determined by measuring the total lipid, protein and carbohydrate content of the test

Table 1 Impact of selected pesticide groups on certain natural enemies.

Pesticide	Biological control agent	Impact	Method	Reference
Organochlorine insectides				
Dieldrin	*Acarina* spp.	Relatively non toxic	Contact toxicity	Edwards and Thompson1973
Lindane	*Sturmiopsis inferens*	Highly toxic	Residual test	Easwaramoorthy *et al*. 1990
	Encarsia sp.; *Aleurodiphilus* sp.	Relatively non toxic	Contact toxicity	Price and Schuster 1991
Endosulfan	*Encarsia* sp.; *Aleurodiphilus* sp.	Harmful	Contact toxicity	Price and Schuster 1991
	Trichogramma cordubensis	Delaying preimaginal development	Residual test	Vieira *et al*. 2001
Organophosphorous and carbamates				
Azinphosmethyl	*Neoseiulus fallacis*	Moderately toxic	Leaf disk	Villanueva and Walgenbach 2005
Azinphosmethyl	*Bassus dimidiator*	Harmful	Residual test	Wilkinson *et al*. 2001
Carbaryl	*Harmonia axyridis*	Harmful	Topical application and insecticide residues	Galvan *et al*. 2006a
Carbaryl+Lindane Sevidol®	*Sturmiopsis inferens*	Harmless	Residual test	Easwaramoorthy *et al*. 1990
Carbofuran	*Sturmiopsis inferens*	Harmless	Soil application	Easwaramoorthy *et al*. 1990
Chlorpyrifos	Phytoseiid mites	Harmless	Foliar application	James *et al*. 2005
	Phytoseiid mites	Harmful	Residue test	Prischmann *et al*. 2005
Chlorpyriphos –ethyl	*Typhlodromus exhilarates* *T. phialatus*	Harmful	Residue test	Barbar *et al.* 2007
Diazinon	*Trissolchus grandis*	Harmful	Topical application	Sheikhi-Garjan 2000
Dimethoate	*Sturmiopsis inferens*	Highly toxic	Residual test	Easwaramoorthy *et al*. 1990
	Micromus tasmaniae	Harmful	Direct application	Booth *et al*. 2007
	Labidura riparia	Harmless	Direct application	Kohno *et al*. 2007
Fenthion	*Trichogramma cacoeciae*	Harmful	Residual test	Grützmacher *et al*. 2004
Heptenophos	*Phytoseiulus persimilis*	Harmless	Residual test	Kavousi and Talebi 2003
Malathion	*Sturmiopsis inferens*	Highly toxic	Residual test	Easwaramoorthy *et al*. 1990
	Bathyplectes curculionissss	Harmful	Residual test	Sabahi *et al*. 2002
Methidathion	*Coccidoxenoides peregrinus*	Harmfull	Direct spray	Wakgari and Giliomee 2003
Methomyl	*Aprostocetus ceroplastae*	Harmful	Direct spray	Wakgari and Giliomee 2001
	Coccidoxenoides peregrinus	Harmful	Direct spray	Wakgari and Giliomee 2003
Methyl-parathion	*Coccidoxenoides peregrinus*	Harmful	Direct spray	Wakgari and Giliomee 2003
Monocrotophos	*Sturmiopsis inferens*	Highly toxic	Residual test	Easwaramoorthy *et al*. 1990
Parathion	*Coccidoxenoides peregrinus*	Harmful	Direct spray	Wakgari and Giliomee 2003
Phosalone	*Bathyplectes curculionissss*	Moderately harmful	Residual test	Sabahi *et al*. 2002
Pirimicarb	*Diaeretiella rapae*	Affects reproductive performance	Contact toxicity	Umoru and Powell 2002
	Diaertiella rapae	Slightly harmful	Direct application	Farag and Gesraha 2007
	Coccinella undecimpunctata	Harmless	Direct spray	Cabral *et al*. 2008
Pirimiphos-methyl	*Phytoseiulus persimilis*	Harmful	Residual test	Kavousi and Talebi 2003
Profenofos	*Coccidoxenoides peregrinus*	Harmful	Direct spray	Wakgari and Giliomee 2003
Prothiofos	*Coccidoxenoides peregrinus*	Harmful	Direct spray	Wakgari and Giliomee 2003
Quinalphos	*Sturmiopsis inferens*	Highly toxic	Residual test	Easwaramoorthy *et al*. 1990
Trichlorfon	*Trichogramma cacoeciae*	Harmful	Residual test	Grützmacher *et al*. 2004
Artificial Pyrethroids				
Bifenthrin	*Harmonia axyridis*	Harmful	Topical application and insecticide residues	Galvan *et al*. 2006a
Cypermethrin	*Pimpla turionellae*	Affects total body weight	Rearing on diet containing sublethal dose	Sak *et al*. 2006
Decamethrin	*Sturmiopsis inferens*	Moderately harmful	Residual test	Easwaramoorthy *et al*. 1990
Deltamethrin	*Trichogramma cordubensis*	Reducing parasitism rate	Indirect spray	Garcia *et al*. 2006
Esfenvalarate	*Encarsia* sp.; *Aleurodiphilus* sp.	Harmful	Contact toxicity	Price and Schuster 1991
	Neoseiulus fallacis	Highly toxic	Leaf disk	Villanueva and Walgenbach 2005
Fenpropathrin	*Neoseiulus fallacis*	Moderately harmful	Residual test	Villanueva and Walgenbach 2005
Fenpropathrin	*Encarsia formosa*	Harmful	Dipping method	Heidari *et al*. 2004
	Neoseiulus fallacis	Moderately toxic	Leaf disk	Villanueva and Walgenbach 2005
Fenvalerate	*Sturmiopsis inferens*	Highly toxic	Residual test	Easwaramoorthy *et al*.1990
Lambda-cyhalothrin	*Micromus tasmaniae*	Toxic	Direct application	Booth *et al*. 2007
	Labidura riparia	Harmless	Direct application	Kohno *et al*. 2007
Permethrin	*Aphidius gifuensis*	Harmful	Residual test	Kobori and Amano 2004
Microbials				
Abamectin	*Trichogramma pretiosum*	Harmful	Residual test	Carvalho *et al*. 2003
	Phytoseiulus persimilis; *Phytoseiulus plumifer*	Harmful	Residual test	Nadimi *et al*. 2008
	Colpoclypeus florus *Trichogramma platneri*	Harmful	Topical application	Brunner *et al*. 2001
	Trichogramma cacoeciae	Harmless	Residual test	Grützmacher *et al*. 2004
Bacillus thuringiensis	*Orius strigicollis*	Harmless	Direct spray	Yoshizawa and Aizawa 2007
	Colpoclypeus florus	Harmless	Residual test	Brunner *et al*. 2001
	Chrysoperla carnea	Increase in mortality, slight decrease in weight	Greenhouse experiments	Dutton *et al*. 2003

Table 1 (Cont.)

Pesticide	Biological control agent	Impact	Method	Reference
	Trichogramma cordubensis	Harmless	Residual test	Vieira *et al.* 2001
Beauveria bassiana	*Labidura riparia*	Harmless	Direct application	Kohno *et al*. 2007
	Neoseiulus californicus	Harmless	Residual test	Castagnoli *et al.* 2005
Emamectin benzoate	*Diadegma semiclausum* *Oomyzus sokolowskii*	Harmful	Contact toxicity	Amano and Haseeb 2005
Milbemectin	*Orius strigicollis*	Harmless	Direct spray	Yoshizawa and Aizawa 2007
Spinosad	*Trichogramma exiguum*	Harmful	Residual test	Charles *et al*. 2000
	Neoseiulus =Amblyseius cucumeris	Harmless	Residue test	Van Driesche *et al*. 2006
	Iphiseius degenerans	Slightly harmful	Residue test	Van Driesche *et al*. 2006
	Harmonia axyridis	Harmless	Topical application and direct spray	Galvan *et al*. 2006b
	Neoseiulus fallacis	Harmful	Leaf disk	Villanueva and Walgenbach 2005
Neonicotinoids				
Acetamiprid	*Neoseiulus fallacis*	Moderately toxic	Leaf disk	Villanueva and Walgenbach 2005
Dinotefuran	*Leptomastix dactylopii*	Harmful	Direct application	Cloyd and Dickinson 2006
	Labidura riparia	Slightly harmless	Direct application	Kohno *et al*. 2007
Imidacloprid	*Colpoclypeus florus*	Highly toxic	Topical application	Brunner *et al*. 2001
	Trichogramma platneri	Highly toxic	Topical application	Brunner *et al*. 2001
	Chrysoperla carnea	Highly toxic	Topical application	Huerta *et al*. 2003
	Tiphia vernalis	Decrease in parasitism	Residual test	Potter and Rogers 2003
	Neoseiulus fallacis	Moderately toxic	Leaf disk	Villanueva and Walgenbach 2005
	Diaertiella rapae	Slightly harmful	Direct application	Farag and Gesraha 2007
Thiamethoxam	*Aphelinus gossypii*; *Delphastus pusillus*	Harmful	Residual test	Torres *et al*. 2003
	10 Genera of carabid beetles *Agonum*, *Amara*, *Anisodactylus*, *Bembidion*, *Chlaenius*, *Harpalus*, *Patrobus*, *Poecilus*, *Pterostichus*, and *Scarites*	Highly toxic	Feeding treated seeds	Mullin *et al.* 2005
	Harmonia axyridis	Harmful	Topical application and insecticide residues	Galvan *et al*. 2006a
	Diaertiella rapae	Slightly harmful	Direct application	Farag and Gesraha 2007
Botanicals				
Acetonic fractions of *Trichilia havanensis*	*Chrysoperla carnea*	Harmless	Topical application	Huerta *et al*. 2003
Azadirachtin	*Trichogramma cacoeciae*	Reduced life table parameters	Residual test	Saber *et al.* 2004
	Amblyseius cucumeris	Harmless	Soil-applied	Thoeming and Poehling 2006
	Hypoaspis aculeifer	Moderately harmful	Soil-applied	Thoeming and Poehling 2006
Extract of neem seed	*Encarsia* sp.; *Aleurodiphilus* sp.	Reduce the parasitoids papulation	Contact toxicity	Price and Schuster 1991
Melia volkensii seed extract	*Chilocorus bipustulatus* var. *iranensis*	Slightly harmful	Insecticide residues	Peveling and Ely 2006
Melia volkensii seed extract	*Pharoscymnus anchorago*	Slightly harmful	Insecticide residues	Peveling and Ely 2006
Natural oil of *Jojoba* plant	*Diaertiella rapae*	Harmless	Direct application	Farag and Gesraha 2007
Natural pyrethrins	*Chrysoperla carnea*	Harmful	Topical application	Huerta *et al.* 2003
Neem	*Campoletic chlorideae*	Slightly harmful	Direct application	Rao *et al*. 2007
NeemAzal®	*Opius chromatomyiae*	Harmless	Soil drench	Babul Hossain and Poehling 2006
Pyrethrins	*Harmonia axyridis*	Highly toxic to first instar, harmless to third instar, pupae, or adults	Direct spray laboratory bioassays	Kraiss and Cullen 2008
Rotenone	*Neoseiulus californicus*	Highly toxic	Residual test	Castagnoli *et al.*2000
IGRs				
Buprofezin	*Orius strigicollis*	Harmless	Direct spray	Yoshizawa and Aizawa 2007
	Coccinella undecimpunctata	Moderately harmful	Direct spray	Cabral *et al*. 2008
Chlorfluazuron	*Aphidius gifuensis*	Harmless	Contact and ingestion toxicities	Kobori and Amano 2004
	Diadegma semiclausum *Oomyzus sokolowskii*	Harmless	Contact toxicity	Amano and Haseeb 2005
Chromafenozide	*Labidura riparia*	Harmless	Direct application	Kohno *et al*. 2007
Cyromazine	*Hemiptarsenus varicornis* *Diglyphus isaea*	Harmless	Contact toxicity	Bjorksten and Robinson 2005
	Orius strigicollis	Harmless	Direct spray	Yoshizawa and Aizawa 2007
Diflubenzuron	*Chrysoperla carnea*	Harmful	Topical application	Medina *et al*. 2003
Etoxazole	*Orius strigicollis*	Harmless	Direct spray	Yoshizawa and Aizawa 2007
Fenoxycarb	*Chrysoperla rufilabris*	Moderately harmful	Topical application	Liu and Chen 2001
	Coccidoxenoides peregrinus	Moderately harmful	Direct spray	Wakgari and Giliomee 2003
Flufenoxuron	*Diadegma semiclausum* *Oomyzus sokolowskii*	Harmless	Contact toxicity	Amano and Haseeb 2005

Table 1 (Cont.)

Pesticide	Biological control agent	Impact	Method	Reference
Lufenuron	*Aphidius gifuensis*	Harmless	Contact and ingestion toxicities	Kobori and Amano 2004
Methoxyfenozide	*Labidura riparia*	Harmless	Direct application	Kohno *et al*. 2007
	Trichogramma cacoeciae	Harmless	Residual test	Grützmacher *et al*. 2004
	Trichogramma cacoeciae	Harmless	Residual test	Grützmacher *et al*. 2004
Pyriproxyfen	*Chrysoperla carnea*	Harmless	Topical application	Medina *et al*. 2003
	Aprostocetus ceroplastae	Harmful	Residual test	Wakgari and Giliomee 2001
	Leptomastix dactylopii	Harmless	Direct application	Cloyd and Dickinson 2006
Tebufenozide	*Chrysoperla carnea*	Harmless	Topical application	Medina *et al*. 2003
Teflubenzuron	*Diadegma semiclausum*	Harmless	Contact toxicity	Amano and Haseeb 2005
	Oomyzus sokolowskii			
Triflumuron	*Aprostocetus ceroplastae*	Slightly harmful	Residual test	Wakgari and Giliomee 2001
	Coccidoxenoides peregrinus	Moderately harmful	Direct spray	Wakgari and Giliomee 2003
Micellaneous classes of pesticides				
Acequinocyl	*Orius strigicollis*	Harmless	Direct spray	Yoshizawa and Aizawa 2007
Amitraz	*Orius albidipennis*	Harmful	Residual test	Ghadamyari and Talebi 2002
Cartap	*Aphidius gifuensis*	Harmful	Residual test	Kobori and Amano 2004
Chlorfenapyr	*Diadegma semiclausum*	Harmful	Contact toxicity	Amano and Haseeb 2005
	Oomyzus sokolowskii			
Dodine	*Trichogramma cacoeciae*	Harmless	Residual test	Grützmacher *et al*. 2004
Fenpyroximate	*Galendromus occidentalis*	Harmful	Residue test	Sáenz-de-Cabezón Irigaray and Zalom 2007
Fipronil	*Hyposoter didymator*	Moderately Harmful	Ingestion toxicity	Morales *et al.* 2004
Flonicamid	*Leptomastix dactylopii*	Harmful	Direct application	Cloyd and Dickinson 2006
Insecticidal soap	*Harmonia axyridis*	Moderately lethal to first and third larvae, no effect on pupae and adults	Direct spray; Laboratory bioassays	Kraiss and Cullen 2008
Mancozeb	*Hemiptarsenus varicornis*	Harmless	Residual test	Bjorksten and Robinson 2005
Mineral oil	*Harmonia axyridis*	Moderately lethal to first and third instars, no effect on pupae and adults	Direct spray; laboratory bioassays	Kraiss and Cullen 2008
	Trichogramma cacoeciae	Moderately harmful	Residual test	Grützmacher *et al*. 2004
Phloxine-B	*Chrysoperla carnea*	Harmless	Topical bioassays	Huerta *et al*. 2003
Propargite	*Chrysoperla carnea*	Harmless	Residual test	Rezaei *et al*. 2007
Pymetrozine	*Orius strigicollis*	Harmless	Direct spray	Yoshizawa and Aizawa 2007
	Chrysoperla carnea	Harmless	Residual test	Rezaei *et al*. 2007
	Coccinella undecimpunctata	Harmless	Direct spray	Cabral *et al*. 2008
Pyridalyl	*Orius strigicollis*	Harmless	Direct spray	Yoshizawa and Aizawa 2007
	Labidura riparia	Harmless	Direct application	Kohno *et al*. 2007
Sulfur	Phytoseiid mites	Moderately harmful	Foliar application	James *et al*. 2005
	Phytoseiid mites	Moderately harmful	Residue test	Prischmann *et al*. 2005
	Trichogramma cacoeciae	Harmful	Residual test	Grützmacher *et al*. 2004
Tebufenpyrad	*Typhlodromus pyri*	Moderately harmful	Residual test	Thwaite *et al*. 1996

organism (DeCoen and Janssen 1997). The ecological relevance of CEA have been demonstrated by comparing with population level parameters such as intrinsic rate of increase (r_m) and net reproductive rate (R_0) (De Coen and Janssen 1997; De Coen and Janssen 2003). Smolders *et al.* (2004) used this technique and concluded that it is a rapid and sensitive measure of the effects of environmental stressors and the results can be linked to effects in the higher levels of biological organization. The CEA methodology has also been used successfully by International Council for the Exploration of the Sea (ICES) (ICES 2006).

As mentioned above, theoretically, one component of CEA methodology (i.e. ETS) is nearly common in all organisms and consequently has been applied for very different taxa; decomposer microorganisms Szabó (2003), marine planktons (Packard *et al.* 1971), different daphnids (Simčič and Brancelj1997), larval stage of chironomids (Simčič 2005) and zebra mussel (Fanslow *et al.* 2001). Likewise, the other component, energy content, can be determined in all organisms.

To date, except for one work in which the differences in energy allocation between brachypterous and macropterous morphs of the pygmy Grasshopper, *Tetrix subulata* were studied (Lock *et al.* 2006), the CEA methodology have been mostly applied for aquatic organisms. This method has been used for non-target organisms other than arthropod biocontrol agents with the objective to environmental risk assessment (De Coen and Janssen 1997; De Coen and Janssen 2003; Smolders *et al.* 2003; Verslycke *et al.* 2004).

Considering some applications of the CEA technique on arthropods (De Coen and Janssen 1997), especially on an insect (Lock *et al*. 2006) we are studying the capability of this method in case of plant pests and their natural enemies as a fast, sensitive and ecologically relevant measure of pesticides' risk to BC agents (unpublished data).

EFFECTS OF DIFFERENT CLASSES OF PESTICIDES ON BIOCONTROL AGENTS

Most of the commonly used insecticides are wide spectrum neurotoxic chemicals which affect target and non-target organisms. Some newly marketed pesticides are reported to be less toxic to natural enemies. Unfortunately many BC agents are susceptible to wide spectrum pesticides. Therefore a potential problem arising from the application of these pesticides is the disruption of beneficial arthropod populations important in BC processes.

In IPM it is important to determine which pesticides are compatible with the major BC agents. The results of some toxicity tests of major classes of pesticides on important BC agents are presented in **Table 1**. These classes include organochlorine, organophosphorus, carbamates, pyrethroids, neonicotinoids, microbials, botanicals and Insect Growth Regulators (IGRs). The effect of pesticides on BC agents

depends on pesticides properties including mode of action, persistence and route to the target (Residual, quasi systemic and systemic action), etc. (Van Emden and Peakall 1996). Each pesticide has its own physical, chemical and biochemical properties and so its effect on a special natural enemy is different from other pesticides. Selective pesticides with low effects on natural enemy provide an opportunity to use them in IPM.

In the Table, different methods and formulations of pesticides are used. Furthermore, the strain and life stage of a natural enemy is important in the classification of pesticide hazard.

CONCLUSION

In order to develop successful IPM programs many studies have been carried out to find out the probable compatibility of pesticides and arthropod biocontrol agents, the two major tools, in controlling plant pest species.

However, differences in the used methods and the measured endpoints make it difficult to compare the results. The traditional laboratory methods have been shown as inefficient leading to unreliable results. International Organization for Biological Control (IOBC) initiated to develop standard methods which are validated and ring tested. These methods make it possible to determine the hazard classes of the toxic compounds. Oomen *et al.* (1991) demonstrated the high validity of the standardized laboratory results by conducting more complex field trials. The methods are being developed and currently there is a growing emphasis on demographic studies which incorporates all lethal and sublethal effects as a summary index so called population growth rate. Life table assays provide more detailed information on the side-effects of pesticides. It has been shown that in contrast to some suggestions the population growth rate is not more sensitive than the individual endpoints. On the other hand the most sensitive endpoints are not necessarily the best and most relevant ones.

Detecting the potential damage to the populations in the early stages would be very helpful in protecting the beneficial insects. This kind of studies is used for some non-target organisms other than the biocontrol agents. The parameters studied are mainly molecular and biochemical changes due to pesticides and other stressors. But, relating the changes in biomarkers to the population level changes is not easy and the probable relations are mainly correlative. However, the scientists who work in this field deeply believe that some day it will be possible. The authors try to find out the possibility of using biomarkers for biocontrol agents.

ACKNOWLEDGEMENTS

We are grateful to Professor Hsin Chi from National Chung Hsing University, Taichung, Taiwan for useful information on life table analysis and Shadieh Gerami for providing data.

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