Natural Product as Avermectins and Milbemycins for Agriculture Perspectives

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Kanchan Bhardwaj, Jayanthi Abraham, and Simran Kaur

Abstract

The very first development of avermectins was done from isolation in Kitasato Institute laboratories, using a novel soil-dwelling bacterium. It was transmittal to the Merck & Co. research laboratory incorporation. These belong to the 16-membered, closely related family, which are macrocyclic lactones constituting of four major and four minor homologous compounds. One of the macrocyclic lactone compounds is milbemycin, which is a group of chemical related to the avermectins and was first isolated from *Streptomyces hygroscopicus* in 1972. They are a group of macrolides chemically associated to the ivermectins. Milbemycin, a commercially available insecticide, consists of milbemycin A3 and milbemycin A4 (30% and 70%, respectively). Milbemycin and avermectin anthelmintic groups share a common action mechanism, but the moxidectin molecular structure differs from avermectin anthelmintics, which afford much potency and high lipid solubility and therefore perseverance. Abamectin is the only compound that belongs to the family avermectin and has some application in crop protection from parasites. Apart from it, abamectin also causes oral and dermal toxicity. Other members of the macrolide group are used in antiparasitic medicines, in order to inhibit the animal products contaminating parasites. Remains of macrocyclic lactones compound including avermectin and milbemycin are used in veterinary medicines to inhibit parasites found in meat and milk.

K. Bhardwaj (✉)
Department of Botany, Shoolini University of Biotechnology and Management Sciences, Solan, Himachal Pradesh, India

J. Abraham
School of Bio Sciences and Technology, Vellore Institute of Technology VIT, Vellore, Tamil Nadu, India

S. Kaur
Department of Biotechnology, Lovely Professional University, Phagwara, Punjab, India

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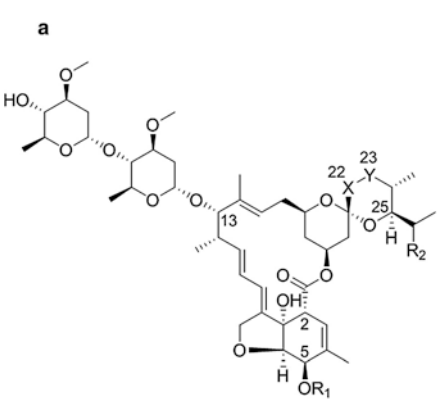
12.1 Introduction

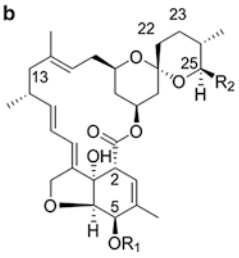
Satoshi Ōmura, a microbiologist, had isolated and cultivated a Gram-positive bacterium (sample NRRL 8165), later identified as an unknown species of *Streptomyces*; the sample was taken from soil and collected from the woods nearby a golf course in Kawana, on the southeast coast of Honshu, Japan. The bacterial isolates were further referred to William Campbell at Merck to check for the antiparasitic activity of the strains (which differed in morphological and culture characteristics). The bioactive compound isolated from the NRRL 8165 cultures shows potent activity against *Nematospiroides dubius* (now known as *Heligmosomoides polygyrus*), which causes mice infection. The purified compound was reported to belong to a macrocyclic lactones (MLs) family. The bioactive compound were so-called avermectins from the bacterium *Streptomyces avermitilis* because of the helminth-free conditions they produced (Burg et al. 1979; Campbell 1981). There are four compound mixtures present in the naturally occurring bioactive compound. There are four compounds consists in the naturally occurring bioactive avermectins. These compounds are A1, A2, B1, and B2, and all of these compounds consist of two variants each, that is, a and b, as shown in Fig. 12.1a (Campbell 1981; Campbell et al. 1983). Due to presence of isopropyl group C25 and chemical structure difference at C22 and C23 position, the bioactivity of avermectins against sheep gastrointestinal nematodes has been proved to have the highest anthelmintic property.

Following the discovery of avermectin, milbemycins were discovered in the year 1973 for protecting crops (Takiguchi et al. 1980). Till 1980s, the ivermectin (IVM) were not used even after these were discovered before. It has been observed that ivermectin and milbemycin helps in preventing infections in dogs (mainly caused by *Dirofilaria immitis*), as it contains bioactivities such as anti-helmintic activity (Rawlings et al. 2001) Sakamoto et al. 1984).

Milbemycin D is commercially being used in Japan only, with the oral dosage 1000 pg/kg for dogs. Milbemycin A has been removed from the Japanese market. From milbemycin, moxidectin (MOX) was formed, which was at molecule C25 at an unsaturated side-chain molecule illustrated in Fig. 12.1b, and was sold in market in year 1990 in Argentina. Moxidectin is being commercialized all over the world as it has a number of applications against cattle parasites in injectable form, wherein the oral therapeutic dosage is maintained at 200 pg/kg for sheep parasites. The milbemycin spectral activity in nematodes is more effective than in arthropods. Apart from it, moxidectin has shown efficacy in both host species, against both ecto- and endoparasites (Ranjan et al. 1992; Webb et al. 1991; Williams et al. 1992). In nematodes, the milbemycin's spectral activity is more effective in comparison to arthropods, and also, license approval of moxidectin proved efficacy against



Avermectins	R_1	R_2	X-Y
A1a	CH_3	CH_2CH_3	CH=CH
A1b	CH_3	CH_3	CH=CH
A2a	CH_3	CH_2CH_3	CH_2-CH(OH)
A2b	CH_3	CH_3	CH_2-CH(OH)
B1a	H	CH_2CH_3	CH=CH
B1b	H	CH_3	CH=CH
B2a	H	CH_2CH_3	CH_2-CH(OH)
B2b	H	CH_3	CH_2.CH(OH)
Ivermectins			
B1a	H	CH_2CH_3	CH_2-CH_2
B1b	H	CH_3	CH_2-CH_2



Milbemycins	R_1	R_2
A3	H	CH_3
A4	H	CH_2CH_3
D	H	$CH(CH_3)_2$
B2	CH_3	CH_3
B3	CH_3	CH_2CH_3
G	CH_3	$CH(CH_3)_2$

Fig. 12.1 The structures of (**a**) avermectins and ivermectins and (**b**) milbemycins. (Adopted from Kim et al. 2017)

endo- and ectoparasites in both host species (Ranjan et al. 1992; Webb et al. 1991; Williams et al. 1992). Since 1990, in USA, milbemycin oxime has also been licensed and used for the prevention of *D. immitis* in dogs and as a healing agent for adult *Ancylostoma caninum*. The amount needed for optimal efficiency is 500 pg/kg (Bowman et al. 1990; Grieve et al. 1989; Stansfield and Hepler 1991). Large doses for dogs have shown safer effects in collies that are sensitive to ivermectin. It's been done by substituting ketoxime at C5 position, which changes position and helps in the reduction of distribution in the central nervous system (CNS) (Shoop and Mrozik 1994; Tranqulli et al. 1991).

12.2 Biosynthesis of Avermectin

Biosynthesis pathway elucidation includes three steps:

1. Initial aglycon (6, 8a-seco-6, 8a-deoxy-5-oxoavermectin aglycons) derived from Polyketide formation.
2. Formation of aglycon to avermectin aglycons.
3. Generation of avermectin by avermectin aglycon's glycosylation.

Table 12.1 Macrocyclic lactones commercially available formulations

Macrocyclic lactones	Formulations
Doramectin	Injectable
Ivermectin	Tablets, oral liquid and paste, injectable, topical
Milbemycin	Tablets
Moxidectin	Tablets, injectable, oral drench, topical
Selamectin	Topical

Adopted from Merola and Eubig (2012)

It has been observed that aglycon moiety of the bioactive avermectins is the result of some fatty acids. In order to generate initial aglycon to form avermectin, alteration in the aliphatic polyketide-derived precursor such as lactonization happened. O-glycosylation happens at positions C13 and C4 using deoxythymidine diphosphate-oleandros, which is converted into avermectins as an end product in the last step of avermectin biosynthesis.

12.3 Formulations

Macrocyclic lactones (MLs) including avermectin and milbemectin are used to kill parasites. These macrocyclic lactones are present in a large number of formulations with different varieties as shown in Table 12.1 (Merola and Eubig 2012). There are some common tablet formulated veterinary products including milbemycin, avermectin, and moxidectin. One of the best examples of macrolide is selamectin (SLM), which is basically used to treat infections caused by heartworm, and also, it is used against endo- and ectoparasites. Some other compounds of avermectin including milbemycin, moxidectin, and doramectin (DRM) help in the treatment of sarcoptic and demodectic mange (Plumb 2005) The symptoms of milbemycin and avermectin intoxication are related to the CNS, for example, blindness, ataxia, depression, mydriasis, and hypertension. If not treated, the symptoms worsen over time.

12.4 Uses in Veterinary and Livestock

Avermectins were firstly developed against onchocerciasis in humans. After some time, it was observed that a number of parasites are being inhibited in humans including enterobiasis, ancylostomiasis, trichuriasis, scabies, head lice, lymphatic filariasis, sea lice, and strongyloidiasis in the presence of avermectins (Canga et al. 2008; Davies and Rodger 2000; Geary 2005; Ottesen and Campbell 1994; Patra 2010). Abamectin (ABM), ivermectin (IVM), doramectin (DRM), and eprinomectin (EPM) all are used against lung nematodes, gastrointestinal nematodes, and cattle ectoparasites. The best proficient route of injecting in relations of availability of drug for livestock as well as some other species is ML subcutaneous injection, in

comparison to topical and oral administration (Gayrard et al. 1999; Laffont et al. 2001; Lespine et al. 2003). ML administrating dosage for animals to pour-on formulations and injectables should be maintained at 0.5 mg/kg and 0.2 mg/kg, respectively. As the ingredient is less absorbable in the gastrointestinal track and skin, and because of that, the dosage required for injections is less than that of the dosage required for pour-on formulations (Bousquet-Melou et al. 2011). Route of administration shows some adverse effects on efficiency of MLs against ectoparasites due to 14–28 days activity of avermectins. For example, injectable or pour-on administration is much effective as compared to oral administration in case of mange mites, while injectable administration is less effective against several red lice, such as *Bovicola bovis* (Benz et al. 1989; Chick et al. 1993). DRM has achieved high activity (for 27 days) (Muniz et al. 1995). Generally, oral administration is best suitable for ruminants that are small. Even though there are rare controls, while EPM is registered for small dairy ruminant's usage, the formulations of cattle are frequently used due to low residues present in milk (Prichard et al. 2012).

IVM is registered for horse use. It is commonly administered as a paste orally or as a liquid oral formulation. IVM has very much efficacy against many nematode parasites that are found on horses as well as bots (Prichard et al. 2012). The IVM and selamectin (SLM) has efficacy against heartworm disease present in cats and dogs. SLM is also effective against lice, fleas, and mange mites. Usually, in heartworm, preventatives are given after 30 days during transmission season of the heartworm. IVM (at 50 μg/kg) act against and kill microfilariae of *Dirofilaria immitis*. IVM take almost 17–37 months for eliminating all different stages of *D. immitis*, including the adult worms (McCall 2005).

Milbemycin derivatives are valuable as agricultural and horticultural acaricidal, anthelmintics, and insecticidal agents (Zhao et al. 2011). Moxidectin (MOX) is basically used to control lung and gastrointestinal nematodes and ectoparasites found in cattle. MOX when given as a single dose has activity for long time based on species type. This is due to MOX's higher persistent efficiency present in the host. Usually, IVM and MOX must not be given to milk-producing dairy cattle, because the milk is excreted very minute. However, in the case of dairy cattle that are lactating, pour-on MOX is registered with no withdrawal of milk, which is due to less toxicity level of MOX as compared to IVM. Injection for long-term lactation of MOX (1 mg/kg) provides 50 days of protection against *Boophilus microplus* (Davey et al. 2011). Generally, MOX commonly shows good effect, when it is taken at recommended dose and dosage to control parasitic resistance development having avermectin as sheep and goats. MOX is also registered and orally given as paste to horses. MOX is highly effective against encysted small strongyle larvae. MOX is also used to eradicate adult stage, as well as the stage that is not mature in the case of hookworms as well as roundworms in cats and dogs, lungworm mites (*Otodectes cynotis*), demodex mites, and sarcoptic mange mites. The MOX injectable preparations provide long-acting defense against *D. immitis* that causes heartworm diseases.

12.5 Uses in Crop Protection

Avermectins were the first reported for their insecticidal activity (Ostlind et al. 1979). Later studies revealed that the avermectins' broad spectrum activity includes its use in agricultural science (Putter et al. 1981). Avermectin B_1 (abamectin) is useful in protecting crop because it is very toxic for arthropods (Dybas et al. 1989). Avermectin B_1 possesses unique effectiveness property in contrary to phytophagous insects, with a value of LC_{90} ranges from 0.02 to 0.24 ppm Eriophyidae members, Tarsonemidae and Tetranychidae (Lasota and Dybas 1991). Avermectin B_1 is deadly poisonous for eriophyid mites (Dybas 1989). It is considerably not as much poisonous to other phytophagous mites, for example, citrus red mite (Dybas 1989). Avermectin B_1's poisonousness to pests and insects are more inconstant. Even though abamectin is more poisonous to tobacco hornworm *Manduca sexta*, tomato pinworm *Keiferia lycopersicella*, Colorado potato beetle *Leptinotarsa decemlineata*, moth diamond back *Plutella xylostella*, budworm *Heliothis virescens*, and *Liriomyza trifolii*, as well as the serpentine leaf miner (LC_{90} values range between 0.02 and 0.19 ppm), it is not much effective against many of Homoptera and Lepidoptera (LC_{90} values range between 1 and > 25 ppm) (Dybas 1989; Lasota and Dybas 1991). Due to its lesser potency against many coleoptera, homoptera and lepidoptera restricted its chances for later development. Even though avermectin B_1 is poisonous to certain aphids, for example, LC_{90} values against *Aphis fabae* are in between 0.2 and 0.5 ppm and also against cotton aphid, *Aphis gossypii*, are in range between 0.4 and 1.5 ppm, avermectin B_1 are not showing the efficacy in regulating aphids in translaminar assays (Dybas and Green 1984; Putter et al. 1981). The efficiency that is reduced at inhibiting aphids is perhaps caused by toxic (or lower) concentrations of avermectin B_1 in phloem tissue where aphids feed actively. Generally, avermectin B_1 is not much toxic to valuable arthropods, particularly when they are introduced after 1 day of application. LC values contrary to many of valuable arthropods are maximum than those for the key target pests (Dybas 1989; Zhang and Sanderson 1990). The spectrum and simultaneous proficiency of emamectin benzoate have not been broadly studied as those for avermectin B_1. Generally, emamectin benzoate is more efficient to a wide-ranging lepidoptera spectrum. It is a highly efficient insect repellent compound registered and developed for use in agricultural practices. The range of LC_{90} emamectin benzoate value, against a lepidoptera family variety, lies between 0.002 and 0.89 ppm (Dybas 1989). Emamectin hydrochloride has high efficacy against armyworm species, for example, beet armyworm *Spodoptera exigua*, than abamectin and is also more potent against southern armyworm *Spodoptera eridania*, than fenvalerate, methomyl, and thiodicarb, respectively (Dybas et al. 1989; Mrozik et al. 1989; Trumble et al. 1987).

Emamectin benzoate is less lethal for different valuable organisms in comparison of abamectin. Emamectin benzoate foliar filtrates were marginally toxic (<20% mortality) to many useful insects, comprising honeybee, and numerous predators and parasitoids, within a day or few hours after application. The reason for its low toxicity is related to the short half-life of emamectin benzoate on foliage. The half-life of foliar dislodgeable residues was likely to be approximately 0.66 days on

celery. After 24 h of application, still 1.3 ng/cm^2 residues were found in celery and alfalfa crops. Emamectin benzoate wet residues are usually toxic in nature to many arthropods; convergent lady beetle *Hippodamia convergens* and common green lacewing *Chrysopa carnea* show tolerance against emamectin benzoate, while they are exposed to wet residues. Emamectin hydrochloride (MK-243) showed slightly adversative effects counter to parasitoids (*Cotesia orobenae* and *Pteromalus puparum*) (Kok et al. 1996).

Milbemectin shows substantial miticidal activity, which affects so many kinds of important mite pest such as the citrus red mite (*Panonychus citri* McGregor), the carmine spider mite (*Tetranychus cinnabarinus* Boisd.), and two-spotted spider mite (*Tetranychus urticae* Koch). Milbemycin D shows insecticidal effects on the gypsy moth *Lymantria dispar* (L). It has also been found that milbemectin shows potential activity in controlling *Bemisia tabaci* population, and its effectiveness is improved by using mineral oils against both whitefly larvae and adults (Pluschkell et al. 1999).

12.6 Mode of Action

The avermectins and milbemycins (A/M) targeted ligand-gated chloride channels. This receptor family is found in both CNS of invertebrates and vertebrates. These receptors pass a number of transmitters. The main transmitter is the one that is gated by glutamate, known as GluCls, and it is known as avermectin (Wolstenholme and Rogers 2005). In invertebrates, the GluCl channels are the only channels found in targeted phyla of avermectin as well as in mollusks. The pharynx of nematode mainly performs a role to intake as well as to process the food partially before it is transferred to the gut. Arrangement of organ differs broadly among the species of nematodes. However, it is considered as the most specific characteristic in their morphology. It includes muscle, gland cells, separate nerve, and other self-regulating system. Pharyngeal pumping is quite sensitive to A/M in nematodes. Apart from it, the values of EC_{50} are also sensitive to the effect caused by drugs, which ranges from 0.2 to 10 nM (Wolstenholme and Rogers 2005). The A/M affects mainly nematodes, and it results in paralysis and ultimately leads to the death of the worm. In the case of nematodes, when GluCl is present on pharyngeal muscle cell, the pumping is inhibited (Fig. 12.2) (Martin 1996). These receptors' irreversible activation in ivermectin causes muscle depolarization, likely due to high internal [Clx] and an ending of pumping (Pemberton et al. 2001). Another major effect caused by A/M on worm is a specious body wall muscle paralysis, which results into immobilization. Though, from evidence point of view it is an indirect effect instead of a direct inhibition of neuromuscular transmission also GluCl indicate no sign in cells of muscle. Nematodes' motion is controlled by both neurons' excitatory and inhibitory motors, which are organized into dorsal and ventral nerve cords. Reciprocal excitation waves and waves of inhibition go down in the body, which lead to relaxation of dorsal muscles and contraction of ventral muscles and vice versa. This causes characteristic sinusoidal swimming motion (Wolstenholme and Rogers 2005). In

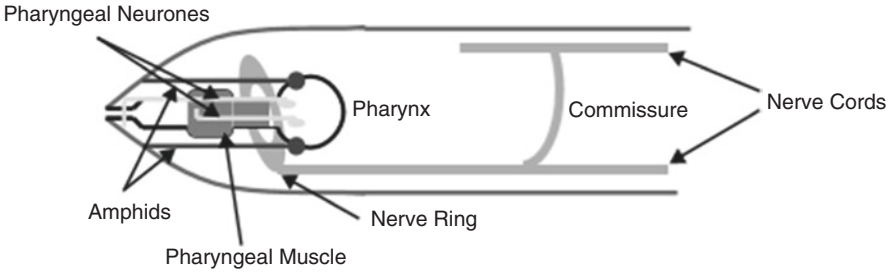


Fig. 12.2 Schematic representation of the distribution of GluCl in nematodes. The cuticle is outlined in gray and the pharynx in black. Structures reported to express GluCl are indicated by arrows. (Adopted from Wolstenholme and Rogers 2005)

Caenorhabditis elegans, studies revealed that interneuron in the head of the worm commands and controlled the motor neurons that regulate the worm's reverse and backward locomotion (Zheng et al. 1999). Initial study reveals that, in *Ascaris suum*, ventral cord avermectin obstructs transmission between interneurons and excitatory motor neurons and also inhibits ventral transmission (Kass et al. 1984). It is expected that the GluCl subunits are expressed on motor neurons, and using reporter gene constructs, it is set in *C. elegans*, while in case of *Haemonchus contortus*, it is set using antibiotics (Fig. 12.2). In *C. elegans*, motor neurons avr-14 and avr-15 are expressed (Dent et al. 1997, 2000). However, the HcGluCla, a3A, a3B, and b subunits all have been identified in *H. contortus* motor neurons (Wolstenholme and Rogers 2005). The anti-GABA antibodies are proposed as motor neuron inhibitors (Portillo et al. 2003). The use of A/M on these inhibitory motor neuron channels would thus apparently result in irreversible hyperpolarization of cell and their consequential incapability to produce action potentials. This avoids inhibition of transmission at the neuromuscular junction and thus waves elimination of muscular relaxation vital for movement.

Even though macrocyclic lactones mainly target glutamate-gated chloride channels, evidence suggest that these drugs, such as moxidectin and ivermectin, can also target cys-loop GABA receptors in *Ascaris*, *C. elegans*, *H. contortus*, and *Trichinella spiralis* causing either a potentiation or receptor activity inhibition (Boisvenue et al. 1983; Brown et al. 2012; Feng et al. 2002; Holden-Dye et al. 1988; Holden- Dye and Walker 1990; Kass et al. 1980; Ros-Moreno et al. 1999).

12.7 Food Contamination

Avermectin family member residues are used against veterinary parasites contained in products that are obtained from animals, for example, meat and milk. The maximum residue limit (MRL) of ivermectin and abamectin in livestock presents as 0.01 mg per kg and 0.005 mg per kg, respectively (Bai and Ogbourne 2016). Ivermectin and abamectin half-life in milk ranges between 4 days and 2 days, respectively (Cerkvenik-Flajs et al. 2007; Imperiale et al. 2004). However, the

presence of abamectin and ivermectin has been observed in milk for 23 days and 1 day, respectively, (Cerkvenik-Flajs et al. 2007; Imperiale et al. 2004). Hence, it is suggested not to use milk and products made from it after 30 days of cattle treatment (Cerkvenik-Flajs et al. 2007). However, withholding period for food products with exposure of abamectin has not been established. As described earlier, it is important to gain approval for abamectin, so that they can be easily used, according to suitable labeling procedures, containing holding period (Moreno et al. 2015).

Avermectin residues in food can be reduced by food processing, though the degree differs under some conditions, e.g., heating milk under low warm conditions at 75 °C and 65 °C for 15 s and 30 min, respectively. Levels of ivermectin did not reduce as they belong to avermectin family, which are lipophilic drugs (Imperiale et al. 2009). Though, in later studies, cheese were obtained from processed milk kept for ripening for 58–61 days, the residues of ivermectin were detected in lower levels, that is, 5–25 days (Cerkvenik et al. 2004). In Europe, a study has been done on beef samples (approx. 1061 beef samples), and around 2.45% of the sample showed detectable veterinary drug residues (0.2–171 μg/kg). However it has been studied that the overall risk of exposure of the European consumer to anthelmintic-drug residues in beef is less than 0.02% which is negligible. These were within the acceptable European maximum residue limits (Cooper et al. 2012). Residues present in meat are capable of lowering up to 50% by boiling or by frying (Slanina et al. 1989).

The maximum residue limit (MRL) acceptable for abamectin in vegetables and fruit is up to 0.01–0.02 mg per kg (Bai and Ogbourne 2016). On the other hand, very less assessment has been done for the assessment of abamectin in food items; nevertheless, abamectin is used to be used as a veterinary treatment or as an acaricide (Kamel et al. 2007; Palmer et al. 1997). Abamectin residue toxicity is found in a number of crops including apricot, celery, Chinese cabbage, cucurbits, and peach. An increase in MRL by 0.05 mg per kg has been reported in certain cases that were investigated by the European Food Safety Authority. However, it was found that the residue presence will not lead to consumer exposure to toxicological reference limits and improbably it was a public health concern (EFSA 2010, 2015). MRL for MOX in milk 40 μg kg^{-1} are established. The maximum residue limit of various citrus fruits for milbemycin A3 is 20 ppb, for milbemycin A4 in pome fruits is 20 ppb, for (Z) 8,9 milbemycin A3 in stone fruits is 20 ppb, and for (Z) 8,9 milbemycin A4 in strawberries is 20 ppb (Food Notice 2018).

12.8 Conclusion

Avermectins and milbemycins are natural products that are further synthesized for marketable supply to use as veterinary therapeutics, pest repellent (insecticides), and pharmacological drugs. These are capable and are utilized for other uses to protect animal, as well as crop, and also in health sector. Other than veterinary and human medicine, there is a lot more about IVM. Although the mode of action of helminth parasite is not yet known, the drug efficacy and host immunity relationship

is worth further study. In the case of food contamination, facts recommend that even the residue of avermectin at high concentrations is enough to cause substantial risk in human health. The main cause of this is short half-life as well as decrement in residue throughout food processing. More important, guidelines that need to be followed on how to carry out residue analysis that requires to develop action mechanism in both nontargeted species and targeted species necessity should be systematically understood.

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