

FEATURED IRAC MEMBER:

Clint Pilcher (DuPont Pioneer) joined the IRAC Plant Biotechnology Team in 2011, and became Team Leader earlier this year. He also represents the Team on the IRAC Steering Group.



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WHITE PAPERS FROM THE IRAC PLANT BIOTECH TEAM

Summary of three white papers covering IRM for transgenic crops in small-holder systems, Industry perspectives on IRM for transgenic crops and IRM for seed blends.

RECENTLY UPDATED IRAC POSTERS

New posters covering insecticide resistance mechanisms for *Myzus persicae* and IRM for *Diaphorina citri*.

RESISTANCE STATUS OF CEREAL APHIDS

A challenge for cereal growers in Northern Europe from pyrethroid resistance in *Sitobion avenae*.

IRM VALUE USING TRAITS AND TRADITIONAL CHEMISTRY

A statement from IRAC International outlining key considerations.

NEWS SNIPPETS & CONFERENCES

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About This Issue

Welcome to another IRAC eConnection Newsletter. As always we try to bring you interesting and informative articles about the work of IRAC and insecticide resistance news from around the world.

In this issue we have summaries of position papers from the Biotechnology Team, details of two updated posters from the Sucking Pest Team on *Myzus persicae* and *Diaphorina citri*, the resistance status of cereal aphids in Northern Europe and a statement from IRAC International on IRM considerations when using both traditional chemistries and traits.

Remember, if you have any news or resistance topics of interest, please let us know us so that we can inform others in the IRAC Network. We hope you enjoy the issue.

IRAC Plant Biotechnology Team White Papers

The IRAC Biotechnology Team recently produced three white papers covering different aspects of insect resistance management for biotech crops which can be downloaded from the IRAC website. Team members summarize the key points from these papers below.

Insect Resistance Management (IRM) for Transgenic Crops in Small-Holder Agricultural Systems

Insects are capable of developing resistance to any pest management tactic, transgenic insect-protected crops are no exception. The consequences of insects developing resistance to transgenic crops will include; loss of revenue to growers due to yield loss, increased costs associated with more aggressive management measures and alteration to crop practices. It is incumbent on technology providers to take proactive measures to delay its onset and develop insect resistance management programs for transgenic crops.

Developing IRM programs in agricultural systems that are dominated by small holders where the economic and practical considerations vary from industrial agricultural systems deserve special consideration. This guide provides an overview of important elements to a proactive IRM program and includes recommendations for IRM in small-holder agriculture systems. These elements include: 1) refuge guidelines, 2) best management practices, 3) education and communication, 4) monitoring, and 5) on-going research.

Critical to small-holder agriculture systems, economic and practical realities are especially important and should complement the scientific basis of any recommended IRM program. Developers must take into account the economic, social and rural agricultural community. In addition, regulators should encourage technology providers to simplify and harmonize IRM programs for similar transgenic products. The full paper can be found at: <http://www.irc-online.org/documents/irm-in-small-holder-systems/?ext=pdf>.

IRAC Plant Biotechnology Team White Papers (Contd.)

Industry Perspectives on Insect Resistance Monitoring for Transgenic Insect-Protected Crops

Resistance monitoring is a fundamental component of insect resistance management (IRM) programs, the goal of which is to maintain product value to customers. This paper provides a consensus of IRAC member views regarding monitoring for insect resistance to transgenic crops. Resistance monitoring provides a means of detecting reductions in susceptibility of target insect pests to insecticides or *Bt* proteins that could reduce the economic value of the product; the primary goal being to detect resistance early enough to effectively deploy resistance mitigation measures and modify the IRM strategy.



Topics in the paper include an overview of properties of a successful resistance monitoring program, what pest species to monitor, where to focus monitoring, laboratory field-based monitoring approaches, appropriate characterization of field collections, and interpretation of bioassay data. The paper closes with a summary of IRAC members' commitment to sustainability and transparency pertaining to resistance monitoring. The full paper can be found at: <http://www.irac-online.org/documents/industry-perspectives-on-ir-monitoring/?ext=pdf>

Seed Blends for Resistance Management of Insect-Protected Transgenic Crops

The benefits and disadvantages of providing "refuge in the bag" are complex and the balance depends on a range of system-specific biological, agricultural, and operational considerations. While blended refuge products assure that growers plant appropriate refuges for their insect-protected biotech crops, movement of larvae between refuge and traited plants can reduce the effective refuge size and increase survival of insects that are heterozygous for resistance alleles. IRAC recognizes that for a blended refuge strategy to be successful for a given pest/crop system, it must be widely adopted among the biotech trait providers. The IRAC Position Paper describes the technical and practical issues that must be considered when evaluating blended refuge and provides guidance on decision making. In general, if a planted refuge is biologically warranted to promote product durability, larval movement between traited and refuge plants is not expected to seriously compromise durability, and grower implementation of a separate refuge is known or expected to be low, seed blends can be a valuable option for refuge deployment. The full paper can be found at: <http://www.irac-online.org/documents/seed-blends-for-irm/?ext=pdf>

Recently updated posters from the IRAC Sucking Pest Working Group

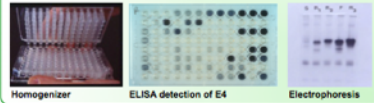
Download the *Myzus persicae* poster: <http://www.ircac-online.org/documents/myzus-persicae-irm-poster/?ext=pdf>

IRAC Major mechanisms of insecticide resistance in green peach aphid *Myzus persicae* Sulzer

Insecticide Resistance Action Committee www.ircac-online.org

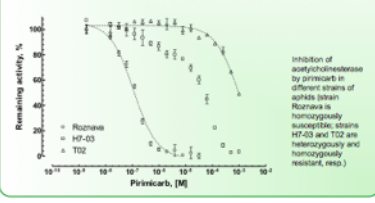
1. Enhanced expression of esterases

- esterases are soluble enzymes hydrolysing ester bonds
- carboxylesterases (E4 and EF4) sequester or degrade esters of organophosphate and carbamate insecticides before they reach their target site
- overproduction of named carboxylesterases causes resistance of *M. persicae* to organophosphates, carbamates, but less to pyrethroids
- detection is done by artificial model substrates or by ELISA
- simple handling and quick determination are further advantages



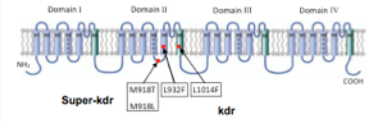
2. MACE (modified acetylcholinesterase)

- carbamates and OP's act by inhibiting acetylcholinesterase (AChE)
- substitution of a serine at position 431 by a phenylalanine in ACE2 leads to target site resistance to dimethylcarbamates, e.g. pirimicarb
- the resistance mechanism is genetically dominant
- resistant aphids are identified with microplate AChE inhibition assays



4. *kdR* (knock-down resistance)

- pyrethroid insecticides cause knock-down resistance ("Kd" or "super kdr"), conferred by changes in a voltage-gated sodium channel protein



- voltage-gated sodium channel in the central nervous system has 4 transmembrane domains with 6 subunits each
- substitution of leucine to phenylalanine results in kdr genotypes, a mutation found in many pyrethroid resistant pest species
- kdR* resistant individuals usually also show high levels of E4 esterase (which contributes to pyrethroid resistance)
- overall effects in *M. persicae* is a loss in fitness

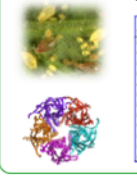
1. Enhanced expression of esterases (cont.)

References:

- Jacobson P & Nauen R (2006) Neonicotinoids: From zero to hero in insecticide chemistry. *PLoS Med* 3(8): e154
- DeGroot-Silva, A. (1998) The evolution of insecticide resistance in the peach-potato aphid, *Myzus persicae*. *Phy. Trans. R. Soc. Lond. B* 353: 1577
- Foster SP et al. (2008) Correlated responses to neonicotinoid insecticides in clones of the peach-potato aphid, *Myzus persicae* (Homoptera: Pemphigidae). *PLoS Med* 5(10): e169
- Nobelskis J et al. (2010) An amino acid substitution in the second acetylcholinesterase in pirimicarb-resistant strains of the peach-potato aphid, *Myzus persicae*. *Biochem Biophys Res Comm* 397: 75
- Nauen R & Denlinger D (2005) Resistance of insect pests to neonicotinoid insecticides. Current status and future prospects. *Arch Insect Biochem Physiol* 58: 200
- Fornace S et al. (2011) Uncommon associations in target resistance among French populations of *Myzus persicae* from almond-rose crops. *PLoS Med* 8(7): e1001081
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3. nAChR target-site resistance

- a single point mutation, R81T in the M. persicae β1-subunit (loop D) of the nAChR confers neonicotinoid resistance
- The R81T mutation confers a loss of direct electrostatic interactions of the electronegative pharmacophore with the basic arginine residue at this key position within loop D



Species	77	78	79	80	81	82	83	84
<i>Homocidus sp.</i> #2	N	V	W	L	T	Q	I	W
<i>Sulphur gall</i> #2	N	V	W	L	T	Q	I	W
<i>Rufus nemorosus</i> #2	N	V	W	L	T	Q	I	W
<i>Acyrthosiphon pisum</i> #1	C	V	W	L	R	L	V	W
<i>Acyrthosiphon pisum</i> #2	N	V	W	L	R	L	V	W
<i>Brevicoryne brassicae</i> #1	N	V	W	L	R	L	V	W
<i>Ulexia nigricornis</i> #1	N	V	W	L	R	L	V	W
<i>Myzodolus cucumeris</i> #1	N	V	W	L	R	L	V	W
<i>Chromopisum rosae</i> #1	N	V	W	L	R	L	V	W
<i>Myzus persicae</i> #2084 #1	N	V	W	L	R	L	V	W
<i>Myzus persicae</i> #2164 #1	N	V	W	L	R	L	V	W
<i>Myzus persicae</i> #RC #1	N	V	W	L	T	L	V	W

Resistance Management Guidelines

- compounds should be used according to the label recommendations
- rotating compounds from different mode of action groups is strongly recommended
- non-chemical control measures should be incorporated (IPM)

IRAC Group	Mode of action	Subgroup	Chemical class
1	Acetylcholinesterase inhibitors	A	Carbamates
		B	Organophosphates
3	Sodium channel modulators	A	Pyrethroids
		B	Neonicotinoids
4	nAChR agonists	C	Sulfofluror
		D	Flupyradfurone
9	Effectors of choriolateral organs	B	Pyrimetozine
		C	Fonicamid
23	Inhibitors of acetyl-CoA carboxylase	None	Spirotetramat
		None	Cytraniliprole

Download the *Diaphorina citri* poster: <http://www.ircac-online.org/documents/diaphorina-citri-irm-poster/?ext=pdf>

IRAC The Asian citrus psyllid, *Diaphorina citri*: 'Insecticide Resistance Management' is the Basis of a Successful IPM Program

Insecticide Resistance Action Committee www.ircac-online.org

Introduction and Biology

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Fig. 1a.), is the insect vector associated with the bacteria *Candidatus Liberobacter asiaticus* and *C. L. americanus*. These bacteria are suspected to be the causal agents of Huanglongbing (HLB) in Asia and America. Trees infected with the bacterial pathogen begin to show symptoms such as early fruit drop and mottled leaves anywhere from 5 months to 3 years after infection. Even during this asymptomatic period, plants can also be source of inoculum, hence the need to manage the vector even if the trees are not showing symptoms (Fig. 1b). Once the trees are infected, their production rapidly declines rendering the infected trees unproductive in a few years.




Fig. 1: (A.) Adult of *D. citri* feeding on a young orange leaf. (B.) HLB-infected trees: asymptomatic (left) and symptomatic (right). Mottled fruits on the ground, leaf coloration, and dieback are more prominent on the symptomatic plant.

Citrus psyllids lay their eggs on the inner-side of unfolding leaves which protect the eggs and early nymphs from adequate insecticide contact, rendering applications of non-systemic insecticides inefficient to manage nymphs. Psyllids develop through 5 nymphal instars, taking between 15 and 47 days to become adults, depending on environmental conditions. Nymphs acquire the bacteria, and the adults vector the disease to uninfected plants and to plants that are already infected. Re-infection increases the bacterial titer in already diseased plants. Adults are considered to be the preferred target for foliar insecticide applications since they vector the bacteria. Systemic soil insecticide target nymphs and adults for the first 2 years after planting, after that period, trees are too big for the current chemistry to be effective.

Resistance to Insecticides

Various levels of insecticide susceptibility have been reported in Florida, USA (Table 1). Although the resistance ratios are not high in comparison to those of other pests, it is important to be vigilant to prevent the onset of resistance for this pest. The results in table 1 are correlated with elevated levels of detoxifying enzymes in both adults and nymphs collected in the field. However, ACP carrying HLB were shown to be more sensitive to insecticides than non-infected psyllids. In Brazil, no tolerance has been reported

	imidacloprid	chlorpyrifos	thiamethoxam	rotation	carbaryl	spinetoram
100% adults	35X	18X	15X	5X	3X	2X
RR50 nymphs	4X	3X	No tested	No tested	3X	6X

Management Plan Example

Figure 2: Management plan and opportunities for MoA rotation used for citrus psyllid based on plant phenology. The rotation uses various MoA which are registered and labeled for control of citrus psyllids. The rotations and number of MoA might vary according to the number of products registered in each country.

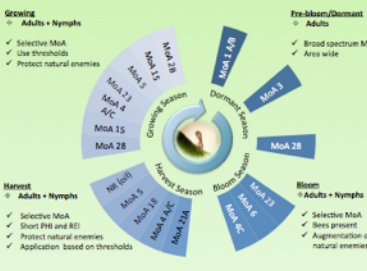


Table 2: Modes of action registered for ACP management. Pest and Resistance management should be based on an appropriate rotation of these MoA

MoA	Mode of action	Subgroup	Chemical class
1 AChE	AChE inhibitors	A	Carbamates
2B	GABA agonists	B	Organophosphates
3	Sodium Channel modulator	A	Pyrethroids
4	nAChR agonist	A	Neonicotinoids
5	nAChR allosteric activators	A	Neonicotinoids
6	Chloride channel activator	C	Sulfofluror
7	Chloride channel activator	D	Flupyradfurone
9	Effectors of choriolateral organs	B	Pyrimetozine
23	Inhibitor of acetyl-CoA carboxylase	C	Fonicamid
28	Ryanodine receptor modulators	None	Spirotetramat
30	Inhibitors of cytosolic biotin synthesis type 0	None	Cytraniliprole
32	Inhibitor of acetyl-CoA carboxylase	None	Spirotetramat
38	Ecdysone receptor agonist	None	Cytraniliprole
39	Mitochondrial complex I electron transport inhib.	None	Cytraniliprole
NR	Horticultural oils	None	Natural products

Integrated ACP Management Guidelines

- Protect nursery plants under netting and use only stock that is certified as HLB-free.
- Transport infected nursery stock according to government regulations.
- Protect young and non-bearing trees with rotation of soil applied systemic insecticides (MoA 4 and MoA 28). In older trees, soil applied systemic insecticides may not work.
- Rotate soil-applied insecticides with foliar sprays of other modes of action. Rotation of different modes of action is key to resistance management.
- Management of adults during dormant season is key to maintain low populations for the rest of the year.
- Use locally defined monitoring methods and intervention thresholds to make spray decisions. Notify manufacturers of any product performance failures immediately.
- Use and protection of bio-control agents is encouraged as part of the IPM programs and to reduce the risk of insecticide resistance development.

Relevant Literature

Pabonierci, A.S. 2013. Bases para o manejo da resistência de *Diaphorina citri* (Homoptera: Liviidae) ao inseticida neonicotinoimidacloprid em pomares de citrus. PhD thesis. Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo. <http://www.teses.usp.br/teses/disponiveis/111/11146/tdc-14952013-162931.pdf>

Rogers, M.E., P.A. Stansly, L.L. Steelman. 2012. 2012 Florida Citrus Pest Management Guide: Asian Citrus Psyllid and Citrus Leaf Miner. IFAS—University of Florida, ENY-794. <http://edis.ifas.ufl.edu/ENY794>

*Tward, S.S., Mene, M.E., Rogers, L.L., Steelman. 2011. Insecticide Resistance in Field Populations of Asian Citrus Psyllid in Florida. Pest Management Science 67: 1238-1248

Vasualoch, P., H. A. Arevalo, A.B. Fraulo, G. Snyder, and P. A. Stansly. 2011. Citrus Greening Bibliographical Database. University of Florida. <http://edis.ifas.ufl.edu/arcgdb>

* Provisional method used by IRAC to evaluate insecticide susceptibility by Asian citrus psyllid

This poster is for educational purposes only. Details are accurate to the best of our knowledge but IRAC and its member companies cannot accept responsibility for how this information is used or interpreted. Advice should always be sought from local experts or advisors and health and safety recommendations followed.

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Pyrethroid resistant grain aphids – a challenge for cereal growers in Northern Europe.

Recent surveys of the grain aphid (*Sitobion avenae*) in the United Kingdom and Ireland have revealed the presence of pyrethroid resistant aphids. If they spread, these resistant aphids could present a new challenge to cereal growers in other parts of Europe.

The grain aphids have been identified as being resistant by an adaption of the sodium channel which forms part of the nervous system in insects and is the site of action of the pyrethroid insecticides. This modification at the target site of pyrethroids is known as the L1014F *kdr* mutation. The mutation is well known in other agricultural and public health pests such as the green peach aphid (*Myzus persicae*) and house fly (*Musca domestica*). What is different to other species is that in this case all the aphids have been found to be heterozygous (single copy) for the resistance allele.

Although the aphids have been demonstrated as having only a relatively low level of resistance to pyrethroid insecticides (up to 40 times less susceptible than insects without the mutation) this shift in sensitivity has been shown to reduce the performance of pyrethroid sprays when the percentage of resistant aphids reach high enough levels. Since their first detection in 2011, resistant aphids have been identified in several English and Irish counties, but the frequency of resistant individuals has not been high enough to cause problems everywhere. Control problems have mainly been focused around Suffolk, Norfolk and Cambridgeshire. Surveys in other European countries have shown that resistant aphids are much rarer in mainland Europe, with only a small number of resistant grain aphids found in parts of Germany and none found in limited surveys of France and Denmark.



The grain aphid is only one of the key species of aphid considered to be pests of cereal crops in Europe. There is currently no indication of pyrethroid resistance in the other species, which include the bird-cherry oat aphid (*Rhopalosiphum padi*), the rose-grain aphid (*Metopolophium dirhodum*) and further eastwards in Europe, the Russian wheat aphid, (*Diuraphis noxia*) and the Spring green aphid (*Schizaphis graminum*).

The resistant grain aphids currently present a challenge to farmers in the UK and Ireland and the concern is that the problem may spread to other areas of Europe. At present, there are few registered insecticides with different modes of action available to farmers (seed treatment or foliar applications) for the control of cereal aphids. This makes it difficult to rotate insecticides with different modes of action, which is the most commonly recommended form of resistance and pest management. In the UK the only other foliar applied insecticides apart from the pyrethroids are organophosphates and carbamates which share the same mode of action (IRAC Group 1). In other countries other insecticide modes of action such as chlordotonal organ modulators (IRAC Group 9) and nicotinic acetylcholine receptor agonists (IRAC Group 4) are available. The situation might get more difficult, if further uses are restricted or insecticides are banned from the market.

If you observe the reduced performance of pyrethroid insecticides against cereal aphids in your region, please work with either your local plant protection organization or pyrethroid manufacturer to determine whether resistance is the cause of the problem and encourage them to report their findings to IRAC.

Resistance management advice for the UK is provided by the Insecticide Resistance Action Group (IRAG) and can be found at www.pesticides.gov.uk/Resources/CRD/Migrated-Resources/Documents/IRAG_Grain_Aphid_Guidance_Sept_2012.pdf, whilst more details on the mechanisms of resistance can be found in: Foster *et al.* A mutation (L1014F) in the voltage-gated sodium channel of the grain aphid, *Sitobion avenae*, is associated with resistance to pyrethroid insecticides. Pest Management Science (2013) DOI 10.1002/ps.3683 (<http://onlinelibrary.wiley.com/doi/10.1002/ps.3683/abstract>)

Links to French and German language versions of this document can be found on the IRAC Sucking Pest Team page at: <http://www.irac-online.org/teams/sucking-pests/>

IRAC International Statement: Considerations for the resistance management value of using insecticidal chemistry on transgenic crops expressing insecticidal proteins.

Chemical insecticides can be applied to conventional and transgenic crops expressing insecticidal proteins. Insecticidal chemistry may be applied to transgenic crops for a number of reasons, particularly to broaden the range of pests controlled or increase the level of target pest control. In certain circumstances, the application of chemical insecticides to transgenic crops also may be considered for insecticide resistance management (IRM) purposes.

All currently commercialized synthetic insecticidal chemistries offer an alternative mode of action to the insecticidal proteins expressed in transgenic plants and there is little evidence for cross-resistance between these chemistries and the insecticidal proteins*. Therefore the combined use of synthetic insecticidal chemicals and proteins which target the same insect pest offers the potential for an IRM tactic that could be beneficial for preserving the susceptibility of the target insects to both components. However, negative IRM impacts may arise if chemical insecticides are applied to a non-transgenic refuge as this reduces the population of insects that are susceptible to the plant expressed protein. Therefore when selecting refuge size and structure, it is important to take into account chemical insecticide application programs.

When considering a pest management program, it is important to take into account IRM considerations for both the transgenic trait (i.e. refuge adoption) and the chemistries being employed (both foliar applied and seed treatments). The following should be considered when assessing the IRM value of applying chemical insecticides to transgenic crops expressing insecticidal proteins:

- 1) An IRM benefit of the combined use of insecticide chemistry and transgenic crops expressing insecticidal proteins will only occur while the target insect population is exposed simultaneously to lethal doses of both the insecticide chemistry and the insecticidal protein(s).
- 2) For there to be an IRM benefit, the insecticide should be applied to the transgenic crop but not the refuge. In cases where both the transgenic crop and the refuge are treated with the insecticide, the IRM benefits will be neutralized. In circumstances where only the refuge is sprayed, this will have a negative effect on IRM for the transgenic crop. Despite the neutral or negative effects on IRM, insecticide sprays applied to the refuge may offer other benefits such as improved pest control.
- 3) In most cases, a refuge-in-a-bag (RIB) strategy does not allow for the selective application of chemical insecticides only to the transgenic plants, and therefore the impact of chemical applications to both the transgenic plants and the embedded refuge is unlikely to provide an IRM benefit.
- 4) The application of insecticides to a field that contains, or is suspected to contain, a significant proportion of target pests that are resistant to the transgenic crop can provide local suppression of the pest population and slow the geographic spread of the resistant insects. This use of insecticides can therefore provide area-wide IRM benefits.
- 5) The combined effects of the chemical insecticide and the expressed insecticidal proteins will be less effective and potentially detrimental if resistance has or is already developing to either the chemical or the protein(s).

*Not including foliar applied sprays which are based on *Bacillus thuringiensis* proteins.



IRAC News Snippets

- ★ A new IRAC Country Team is in the process of being set up in Argentina working in conjunction with the industry association CASAFE.
- ★ The Arthropod Pesticide Resistance Database which is maintained by the Michigan State University with support from IRAC, is in the process of being updated with a new platform, improving data entry, usability and search functionality.
- ★ The IRAC Coleoptera Working Group is in the process of collecting and collating the pollen beetle monitoring results for 2014. The results will be available in early 2015 and presented in a poster format as per previous years.

Conferences & Symposia

- ★ ASTMH 63rd Annual Meeting, New Orleans, Louisiana, Nov. 2-6, 2014
- ★ 62nd ESA Annual Meeting, Portland, Oregon, Nov. 16-19, 2014
- ★ 8th International IPM Symposium, Salt Lake City, Utah, March 23-26, 2015
- ★ Resistance 2015, 7th Intl. Meeting, Rothamsted, UK, Sept 14-16, 2015

Links to the websites for the conferences and symposia can be found on the IRAC Events Page at: <http://www.irac-online.org/events/>

Feedback

The eConnection is prepared by the IRAC International Communication & Education Working Group and supported by the 13 member companies of the IRAC Executive. If you have information for inclusion in the next issue of eConnection or feedback on this issue please email aporter@intraspin.com

Disclaimer

The Insecticide Resistance Action Committee (IRAC) is a specialist technical group of CropLife. Information presented in this newsletter is accurate to the best of our knowledge but IRAC and its member companies cannot accept responsibility for how this information is used or interpreted. Advice should always be sought from local experts or advisors and health and safety recommendations followed.

FURTHER INFORMATION

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Alan Porter, IRAC Coordinator

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