Sublethal Effects of Insecticidal Substances in Plant Protection Products or Genetically Modified Plants on Non-Target Invertebrates

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Introduction

- Genetically modified plants (GMPs) with insect resistance produce insecticides over the full life-time.
- Systemic insecticides are designed to protect the crop over prolonged time periods.
- Thus, in both cases, non-target organisms can be chronically exposed, partly in low doses.
- This type of exposure may lead to sublethal rather than lethal effects.
- We evaluated for which invertebrate taxa which sublethal endpoints are addressed in the first tier effect assessment of plant protection products (PPP) in the EU and compared the outcome with current practice in the effect assessment of GMP having insecticidal properties and propose that both risk assessment frameworks would benefit from each other.

Testing terrestrial invertebrates in the risk assessment of plant protection products (PPP)

- Data requirements are defined in EU Reg 283/2013 [1]:
  - Soil invertebrates (earthworms, springtails and mites) are tested with the test item mixed into soil.
  - Non-target arthropods, represented by a predatory mite and a parasitoid wasp, are tested by exposure via contact.
  - Similar test protocols]2] exist for seven additional 'beneficial' non-target arthropods.
  - Bees are tested for exposure via contact and diet.
- For the soil organisms, effects on reproduction are routinely tested.
- For the standard non-target arthropods (parasitoid wasp and the predatory mite), tier 1 tests usually focus on lethal effects but the protocols allow also testing effects on reproduction.
- For bees, effects on reproduction cannot be tested in laboratory experiments but a protocol on development of larvae is available.

Test protocols for terrestrial invertebrates listed in the data requirements for active substances [1] of PPP plus IOBC protocols [2] of additional species

Species	Group	Function	Exposure path	Duration [d]	Su/ Im	De	Gr	Re	Be	OECD TG
Eisenia fetida / E. andrei	Oligochaeta	Decomposer	soil	56	x		X	x		222
Folsomia candida / F. fimetaria	Collembola	Decomposer	soil	21-28	x			x		232
Hypoaspis aculeifer	Arachnida	Predator	soil	14	x			x		226
Apis melifera	Hymenoptera	Pollinator	diet	2-4	x					213
Apis melifera	Hymenoptera	Pollinator	overspray	2-4	x					214
Apis melifera	Hymenoptera	Pollinator	diet	10	x				x	245
Apis melifera	Hymenoptera	Pollinator	diet	22	x		x			GD 239
Aphidius rhopalosiphi	Hymenoptera	Parasitoid	contact	ca. 13	x			x		IOBC
Typhlodromus pyri	Acari	Predator	contact	14	x			x		IOBC
Aleochara bilineata	Coleoptera	Predator	contact	28	x			x	x	IOBC*
Chrysoperla carnea	Neuroptera	Predator	contact	35 - 42	x			x		IOBC*
Coccinella septempunctata	Coleoptera	Predator	contact	> 28	x			x		IOBC*
Orius laevigatus	Heteroptera	Predator	contact	ca. 21	x			x		IOBC*
Pardosa sp.	Araneae	Predator	contact, overspray	14 - 21	x				x	IOBC*
Poecilus cupreus	Coleoptera	Predator	contact, overspray	≥ 14	x				x	IOBC*
Trichogramma cacoeciae	Hymenoptera	Parasitoid	contact	≥13	x			x		IOBC*

Su: survival, Im: immobilisation, Re: reproduction, Gr: growth, De: development, Be: behaviour
TG: OECD Technical Guidance, GD: OECD Guidance document, IOBC: Candolfi et al. (2000) [2]

Testing terrestrial invertebrates in the risk assessment of GMP

- No data requirements are defined - selection of test species and test protocols is part of the problem formulation conducted by the notifier.
- To get an overview on current practice, the Tier 1 tests of the six GMPs reviewed by Roberts et al. (2020) [3] were analysed:
  - Test protocols used for PPP are modified to address exposure via diet, either via the isolated toxin mixed into diet or via GMP material.
  - Survival is almost always assessed but growth and/or development are also often monitored.
  - Analysis of reproduction is much rarer, but this may be partly explained by the mode of action of most GMP assessed so far.
  - Often the standard test species used for PPP ERA are tested. Depending on the GMP additional taxa, more related to the pest, are tested (e.g. butterflies).
  - For one active substance (Cry3Bb1 targeting Chrysomelidae) 31 Coleopteran species have been tested explaining the dominance of this group in the data set.
  - Eight terrestrial invertebrate species were tested in at least every second example, including the standard test species for earthworms, spring tails, the honey bee and predatory or parasitoid arthropods covered in the IOBC guidelines. In the US, the lady beetle *Coleomegilla maculata* is often tested in addition.

Conclusions

- Gaps exist in the lower tier PPP and GMP ecological risk assessment (ERA) for non-target invertebrates with respect to
  - exposure via food (PPP),
  - consideration of sublethal effects (PPP and GMP),
  - test protocols for species representing taxonomic groups and ecological functions not sufficiently represented yet (PPP and GMP).
- IOBC test guidelines for 9 non-target arthropods [2] have been used for over 20 years now in PPP ERA for testing contact and overspray exposure but also, with modifications, to consider exposure via diet in GMP ERA
- IOBC guidelines [2] cover only non-target arthropods, which are predators or parasitoids of pest species.
- Pollinators are represented by testing honeybees and, more recently, also bumble bees and solitary bees [4] .
- Lepidoptera are already identified to be highly relevant for PPP lower tier assessment [5] but a test protocol is still not available while non-target lepidopteran species have been tested already for GMP producing toxins against lepidopteran pests. However, standardisation is missing [6].
- Thus, isn't it time for a cooperative action of scientists from both, the PPP and the GMP community, to collect experiences gained in the past with different test protocols and to initiate the preparation of refined or new test guidelines for non-target arthropods to better reflect sublethal effects, exposure via diet and, in consequence, effects on biodiversity?

References

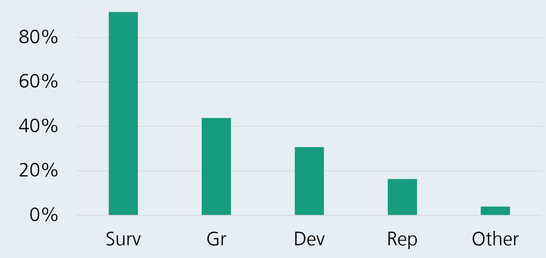
[1] Commission Regulation (EU) No 283/2013. OJ L 93, 3.4.2013. [2] Candolfi MP et al. (2000): Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC/WPRS. [3] Roberts et al. (2020): doi:10.3389/fbioe.2020.00556. [4] EFSA (2023): doi:10.2903/j.efsa.2023.7989. [5] EFSA PPR panel (2015): doi:10.2903/j.efsa.2015.3996. [6] Lang et al. (2019): doi:10.1186/s12302-019-0220-2

Endpoints assessed in Tier 1 tests for 6 insecticidal substances produced by GMPs reviewed in [3]

Active substance Reference*	Target pest group(s)	NTI tests	Sur	Gro	Dev	Rep	Beh
Cry51Aa2.834 (Bachman et al. 2017)	Hemiptera, Thysanoptera	14	14	2	3	1	0
Cry34/Cry35Ab1 (Schrijver et al. 2016)	Chrysomelidae (Coleoptera)	25	25	13	8	3	1
mCry3A (Raybould et al. 2007)	Chrysomelidae (Coleoptera)	6	4		2	1	0
Cry3Bb1 (Devos et al. 2012)	Chrysomelidae (Coleoptera)	89	79	46	29	14	5
Vip3A (Raybould & Vlachos 2011)	Lepidoptera	9	8	2	1	3	0
DvSnf7 RNA (Bachman et al. 2016)	Chrysomelidae (Coleoptera)	10	10	4	4	3	0

Sur: survival, Rep: reproduction, Gro: growth, Dev: development, Beh: behaviour
* for references see [3]

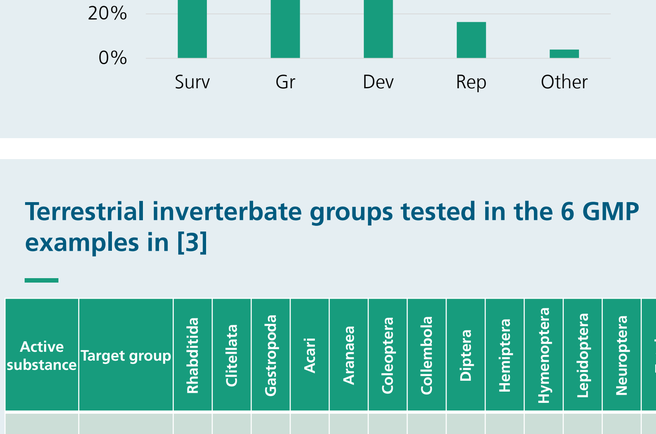
Proportion of tests addressing different endpoints



Terrestrial inverterbate groups tested in the 6 GMP examples in [3]

Active substance	Target group	Rhabditida	Citellata	Gastropoda	Acari	Araneae	Coleoptera	Collembola	Diptera	Hemiptera	Hymenoptera	Lepidoptera	Neuroptera	Total
Cry51Aa2.834_16	Hemiptera, Thysanoptera	0	1	0	0	0	5	1	0	1	2	4	0	14
Cry34/Cry35Ab1	Coleoptera	0	1	0	0	0	5	1	0	1	2	3	1	14
mCry3A	Coleoptera	0	1	0	0	0	3	0	0	1	1	0	0	6
Cry3Bb1	Coleoptera	1	3	3	1	1	31	1	2	3	2	3	1	52
Vip3A	Lepidoptera	0	1	0	0	0	3	1	0	1	1	0	1	8
DvSnf7 RNA	Coleoptera	0	1	0	0	0	3	1	0	1	2	0	1	9
Total number of test species		1	4	3	1	1	37	1	2	3	3	7	1	64

Test species used in at least 3 of the 6 examples



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