Herbeoordeling thiacloprid en acetamiprid– eerste fase

5.1.2.e *en* 5.1.2.e

September 2013

Doel notitie

Het doel van deze notitie is het College te adviseren over het vervolgtraject van het project 'herbeoordeling risico voor bijen van thiacloprid en acetamiprid". De eerste fase van het project is afgerond waarin een evaluatie van nieuwe aangeleverde studies is gemaakt. Op basis van de nieuwe informatie is een analyse gemaakt of er nieuwe wetenschappelijke inzichten zijn die aanleiding geven voor een herbeoordeling van de middelen of een alternatief vervolgtraject.

Aanleiding

Tijdens het AO bijensterfte op 16 mei 2013 heeft de staatssecretaris de Tweede Kamer toegezegd het Ctgb te vragen thiacloprid en acetamiprid nationaal versneld te bezien met het oog op het risico voor bijen. Thiacloprid en acetamiprid zijn neonicotinoïden, stoffen die zeer toxisch kunnen zijn voor bijen. Voor de neonicotinoïden imidacloprid, thiamethoxam en clothianidine is door EFSA een herbeoordeling uitgevoerd. Er is op basis van deze herbeoordeling door EFSA door de EC besloten om aanvullende restricties en inperking van toepassingen communautair op te leggen voor gebruik van deze middelen om het risico naar bijen te minimaliseren. Thiacloprid en acetamiprid zijn acuut minder giftig voor bijen dan thiamethoxam, imidacloprid en clothianidine. Door de lagere acute toxiciteit is besloten dat deze stoffen later in het herbeoordelingtraject worden beoordeeld door EFSA. Echter, de commissie heeft besloten om de herbeoordeling van thiacloprid en acetamiprid voor bijen door EFSA niet uit te laten voeren vanwege de hoge werkdruk bij EFSA.

Doel project

Het doel van het project is concrete risico's van middelen op basis van thiacloprid en acetamiprid voor bijen aanpakken. Het betreft 7 middelen op basis van thiacloprid en 1 middel op basis van acetamiprid. Het project heeft 2 fases: eerste fase is een evaluatie van nieuwe studies en een analyse of er nieuwe wetenschappelijke inzichten die aanleiding geven voor een herbeoordeling van de middelen. Fase II zal de herbeoordeling van de middelen zijn, indien nodig.

Nieuwe gegevens

In juni 2013 heeft het Ctgb de toelatinghouders, deskundigen en NGO's uitgenodigd om voor de herbeoordeling van thiacloprid en acetamiprid alle relevante data aan te leveren. Hierop is reactie gekomen van de toelatinghouders van thiacloprid en acetamiprid, Bayer CropScience en Nisso Chemical Europe (p/a Certis Europe). Er is daarnaast reactie gekomen van Denka Registrations, Greenpeace, PAN Europe, 5.1.2.e en 5.1.2.e Aanvullend hierop heeft het RIVM in opdracht van Ctgb een literatuuronderzoek uitgevoerd. Een overzicht van alle ingeleverde studies is te vinden in bijlage I.

Analyse van nieuwe gegevens

Er is een eerste screening uitgevoerd van de geleverde gegevens. Er zijn veel nieuwe studies beschikbaar, zowel acute studies als semi-veldstudies en veldstudies naar de effecten op honingbijen van thiacloprid of acetamiprid, al dan niet met gelijktijdige blootstelling aan andere bestrijdingsmiddelen (fungiciden). Het grootste deel van de beschikbare gegevens is niet meegenomen in de Europese stofbeoordeling van thiacloprid en acetamiprid. De EU stofbeoordeling is slechts gebaseerd op enkele acute toxiciteitstudies en een enkele semi-veldstudie.

Uit de screening van de geleverde gegevens blijkt dat synergisme kan optreden tussen enerzijds thiacloprid/ acetamiprid en anderzijds fungiciden / piperonyl-butoxide (PBO), waarbij de toxiciteit van thiacloprid en acetamiprid met een factor 10 - 1000 toeneemt, zie

tabellen 1 en 2. Met een verhoging van een factor 1000x, wordt de geobserveerde LD_{50} van dezelfde orde van grootte als de acute toxiciteit van imidacloprid.

Echter, er zijn ook studies door Bayer Cropscience aangeleverd, vijf semi-veldstudies voor thiacloprid, waarbij een synergistische interactie niet lijkt op te treden bij een combinatie van een neonicotinoïde en fungicide, zie tabel 3. De combinaties die in Iwasa et al. (2004) de sterkste interacties geven, zijn niet onderzocht in (semi-)veldstudies. Wel heeft Iwasa (2004) een (zeer summier beschreven) aged residue proef gedaan met honingbijen in kooitjes, blootgesteld aan alfalfaplanten die 3 of 24 uur eerder waren bespoten met een tankmix van acetamiprid en triflumizole. In deze proef werd geen verschil in toxiciteit geconstateerd met de controle noch met acetamiprid alleen.

Nisso Chemical Europe heeft geen aanvullende studies aangeleverd aangaande synergisme voor acetamiprid.

Een uitgebreide analyse van de studies is noodzakelijk om te bepalen in welke mate de studies kunnen worden geëxtrapoleerd naar de toepassingen die zijn toegelaten op de Nederlandse markt voor thiacloprid en acetamiprid.

Table 1. Acute contact toxicity values (24h) of neonicotinoid insecticides without and with pre-treatment with 10 µg/bee other substance one hour before insecticide. Pre-treatment with some substances increases the toxicity of acetamiprid and thiacloprid (but not imidacloprid) with a factor of 100-1000. Source: Iwasa et al. 2004.

Table 2
Pretreatment effect of general insecticide synergists, DMI-fungicides, and a plant growth regulator on honey bee toxicity of neonicotinoid insecticides

Insecticide synergist[a]	n[b]	LD_{50} (µg/bee)[c]	95% CI[d]	Chi-square	Slope ± SE	SR[e]	95% CI[e]
Acetamiprid							
Alone	465	7.07	4.57–11.2	0.826	1.77 ± 0.105	1	
PBO	202	1.17	0.342–3.79	1.18	1.55 ± 0.181	6.04	4.29–8.51
DEF	124	2.39	0.278–12.4	5.85	2.96 ± 0.736	2.96	1.83–4.76
DEM	123	6.94	4.10–13.2	0.278	1.46 ± 0.140	1.02	0.783–1.33
Triflumizole	215	0.0290	0.0080–0.102	3.46	1.91 ± 0.240	244	171–347
Propiconazole	201	0.0675	0.0231–0.197	2.63	2.30 ± 0.242	105	76.7–143
Triadimefon	131	0.0844	0.0431–0.176	0.693	2.05 ± 0.198	83.8	64.2–110
Epoxiconazole	156	0.500	0.156–1.66	4.42	2.74 ± 0.404	14.1	10.0–20.0
Uniconazole-P	156	1.12	0.270–4.96	3.66	2.05 ± 0.349	6.31	4.22–9.45
Imidacloprid							
Alone	137	0.0179	0.0092–0.0315	0.303	1.70 ± 0.176	1	
PBO	152	0.0105	0.0061–0.0172	0.0889	1.66 ± 0.112	1.70	1.29–2.26
Triflumizole	125	0.0097	0.0052–0.0168	0.694	2.76 ± 0.284	1.85	1.67–3.09
Propiconazole	145	0.0118	0.0038–0.0303	1.01	2.12 ± 0.272	1.52	1.04–2.24
Thiacloprid							
Alone	158	14.6	9.53–25.4	0.480	2.73 ± 0.371	1	
PBO	193	0.0948	0.0406–0.211	0.424	1.64 ± 0.134	154	115–207
Triflumizole	160	0.0128	0.0031–0.0415	1.66	2.32 ± 0.363	1141	752–1740
Propiconazole	159	0.0261	0.0083–0.0690	1.05	2.27 ± 0.298	559	388–811

[a] In all, 10 µg of synergist was applied to the dorsal thorax of each worker honey bee 1 h prior to insecticide application.
[b] Number of insects tested.
[c] Results were corrected for control mortality. Dose is given in micrograms of active ingredient.
[d] CI, confidence interval.
[e] SR, synergism ratio (the LD_{50} of insecticide alone/LD_{50} of synergist and the insecticide).

Table 2. Acute oral and contact toxicity values (48h) of a mixture of thiacloprid (THIA) and one or two fungicides. Source: three studies by 5.1.2.e (2003, from Bayer dossier). Simultaneous application of tebuconazole, but not prothioconazole, increases the toxicity of thiacloprid with a factor of ca. 10.

test substance	LD50 oral	*factor increase in toxicity*	LD50 contact	*factor increase in toxicity*	Dose range other substance
THIA	17*		41*		
THIA + tebuconazole	2.5	*6.8*	3.7	*11*	2.3-36 µg/bee
THIA + prothioconazole	16.3	*1.0*	15.6	*2.6*	1.6-25.4 µg/bee
THIA + tebuconazole + prothioconazole	1.6	*11*	5.3	*7.7*	0.4-7.1 µg/bee

*This value is taken from the LoEP of thiacloprid and used for comparison with the results of the 5.1.2.e (2003) studies since these did not determine the toxicity of THIA alone. This may over- or underestimate the increase in toxicity.

Table 3. Results of semi-field studies with thiacloprid in tankmix with fungicides. All studies from Bayer Cropscience dossier.

Source	Test dose & application	*Results*	*Remarks*
5.1.2.e & 5.1.2.e *2003*	Tankmix of 96 g thiacloprid/ha and 175 g prothioconazole/ha. Application during active foraging on full-flowering summer rape.	No increased mortality, effects on flight intensity, pollinating activity, number of bees in hive, brood development, food storage area and behaviour.	Exposure duration in cage was 7 days. The bees were monitored for one full brood cycle.
5.1.2.e & 5.1.2.e *2003*	Tankmix of 96 g thiacloprid/ha, 125 g prothioconazole/ha and 125 g tebuconazole/ha. Application during active foraging on full-flowering summer rape.	No increased mortality, effects on flight intensity, pollinating activity, number of bees in hive, brood development, food storage area and behaviour.	Exposure duration in cage was 7 days. The bees were monitored for one full brood cycle.
5.1.2.e 5.1.2.e & 5.1.2.e *2006*	Tankmix of 72 g thiacloprid/ha and 375 g tebuconazole/ha,. Application during active foraging on full-flowering Phacelia.	No increased mortality, effects on flight intensity, pollinating activity, number of bees in hive, brood development, food storage area and behaviour.	Exposure duration in cage was 8 days. The bees were monitored for one full brood cycle. Non-GLP study (can only be considered as supplemental information).
5.1.2.e 5.1.2.e & 5.1.2.e *2006 (3 studies).*	Tankmix of 72 g thiacloprid/ha, 175 g prothioconazole/ha and 5 g lambda-cyhalthrin/ha. Application during active foraging on full-flowering Phacelia.	No increased mortality, effects on flight intensity, pollinating activity, number of bees in hive, brood development, food storage area and behaviour.	Exposure duration in cage was 8 days. The bees were monitored for one full brood cycle. Non-GLP study (can only be considered as supplemental information).

Source	Test dose & application	*Results*	*Remarks*
5.1.2.e 5.1.2.e & 5.1.2.e *2006 (3 studies).*	Tankmix of 72 g thiacloprid/ha, 175 g prothioconazole/ha and 7.5 g alpha-cypermethrin/ha. Application during active foraging on full-flowering Phacelia.	No increased mortality, effects on flight intensity, pollinating activity, number of bees in hive, brood development, food storage area and behaviour.	Exposure duration in cage was 8 days. The bees were monitored for one full brood cycle. Non-GLP study (can only be considered as supplemental information).

Synergisme met fungiciden is niet aan de orde geweest in de Europese beoordeling en is tot nu toe niet meegenomen bij de beoordeling van de Nederlandse toelatingen. Mogelijk is hierdoor de toxiciteit van thiacloprid en acetamiprid in de praktijk onderschat. Het is dus noodzakelijk om de aanwijzingen voor het optreden van synergisme verder te onderzoeken. Er is een herziene risicobeoordeling nodig waarbij gekeken wordt naar de relevantie van de geleverde gegevens voor de in Nederland toegelaten toepassingen en naar de gangbare landbouwpraktijk. Hierbij valt te denken aan voorgeschreven tankmixen op WGs, maar ook aan overlap van de toegelaten toepassingen van de individuele middelen op basis van de neonicotinoïden en fungiciden. Mogelijke gelijktijdige blootstelling wordt bijvoorbeeld bevestigd in studies zoals Pettis et al. (2013), waar in verschillende gewassen een cocktail van pesticiden in pollen wordt aangetoond, inclusief triazolen en thiacloprid en acetamiprid (zie Bijlage II voor overzichtstabel).

Overige insecticiden en synergisme.
Bij de herbeoordeling van de risico's voor bijen van de neonicotinoïden thiamethoxam, clothianidine en imidacloprid door EFSA in 2012 is synergisme met fungiciden niet specifiek meegenomen. Er is voor het huidige project niet specifiek naar literatuur gezocht, maar er is één studie bekend waar de interactie van imidacloprid en triazolen en PBO is getest aangaande de toxiciteit voor bijen. Uit deze studie blijkt dat er geen synergisme optreedt tussen de stoffen bij de geteste combinatie concentraties (zie tabel 1). Indien gewenst kan dit verder onderzocht worden in de tweede fase van de herbeoordeling.

Synergisme is meegenomen in de EFSA scientific contribution, waar specifieke stofgroepen geïdentificeerd zijn voor synergistische interacties, zie bijlage II. Hieruit blijkt dat neonicotinoïdes niet de enige groep stoffen waar synergisme optreedt met triazolen.

Synergisme wordt niet meegenomen in risicobeoordelingen, zelfs als bekend is dat er wetenschappelijke studies zijn waarin synergistische interacties tussen stoffen wordt aangetoond.

Aandachtspunten NGOs
Op 24 juli en 16 september 2013 heeft overleg plaatsgevonden met NGOs, deskundigen en industrie. Op deze bijeenkomsten zijn aandachtspunten aangedragen. Bij de eerste bijeenkomst waren er 3 specifieke aandachtspunten, zijnde:
- *Neem het voorzorgsprincipe als specifiek onderdeel mee in de herbeoordeling van thiacloprid en acetamiprid.*

Het voorzorgsprincipe kan in overweging worden genomen indien een concreet risico is vastgesteld. Hierdoor dient het voorzorgsprincipe in fase II in acht te worden genomen.

- *In fase I waar WUR en RIVM als kennispartners optreden zouden meer NGOs/deskundigen moeten worden meegenomen.*

In fase I zijn de studies geëvalueerd door het RIVM omdat dit een EI is met ASL. Voor de notitie is er beperkt gebruik gemaakt van de kennispartners omdat het potentiële probleem vrij helder te identificeren was. Het ligt in de lijn der verwachting dat bij de tussenfase en fase

II er veel wetenschappelijk discussies zullen zijn rondom synergisme en extrapolaties van de studies naar een risicobeoordeling. Een 3e kennispartner voor synergisme is wenselijk.

- *Benoem duidelijk het kader van beoordeling: wordt alleen de honingbij of worden alle wilde bijen meegenomen in de herbeoordeling.*

Het overgrote merendeel van de studies zijn studies met de honingbij. Slechts een beperkt aantal studies is ingeleverd voor hommels en wilde bijen, en dan ook enkele met soorten uit niet-Europese regionen. Het kader wordt nu ingeschat dat het betrekking heeft op de honingbij. Bij fase II zal dit worden vermeld. Tijdens de bijeenkomst met de NGOs en deskundigen is ook aangegeven dat het overgrote merendeel van de studies de honingbij betrof. Overige aandachtspunten zijn te vinden in bijlage III.

Op 16 september zijn de volgende aandachtspunten aangedragen:

- 5.1.2.e heeft aangegeven dat synergisme voor alle middelen en actieve stoffen zou moeten worden meegenomen in de beoordelingen.
- Denka Registrations B.V. heeft geen specifieke aandachtspunten aangegeven maar was zeer geïnteresseerd in de algemene problematiek, ontwikkeling van het richtsnoer en welke scope het College de herbeoordeling eventueel zou gaan geven, indien deze richting zou worden gekozen. Na een discussie over de nieuw EFSA bijenguidance was zijn devies dat ongeacht wat het College besluit, er altijd een 'bij-vriendelijk' middel mee moet lopen om de selectiviteit van de beoordelingscriteria te toetsen. Denka heeft middelen o.b.v. pyrethrines waar synergisme met fungiciden ook in enkele studies is aangetoond.

Analyse van probleem en Advies aan het College

potentieel probleem vs concreet risico

In fase I is synergisme geïdentificeerd als potentiële grond voor onderschatting van het risico voor bijen in de huidige toelatingsbeoordeling voor middelen op basis van thiacloprid en acetamiprid. De overige geleverde informatie gaat onder andere in op metabolieten, chronische toxiciteit in het laboratorium, (semi-)veldtoxiciteit van de individuele stoffen, generieke monitoring en risicobeoordelingsmethodiek (inclusief mogelijke interactie met parasieten). Deze informatie geeft geen directe aanleiding tot herbeoordeling van thiacloprid en acetamiprid (een jaar voorafgaand aan de herregistratie).

Op dit moment is echter weinig inzage in de orde van grootte van de potentiële onderschatting van het risico van bijen door synergisme. Deze onderschatting van het risico van bijen zal concreet, of zo concreet mogelijk, moeten worden gemaakt om de vervolgstappen degelijk en transparant te onderbouwen. Ook bij de discussie met NGOs, deskundigen en politiek is het noodzakelijk om meer inzage in de bredere problematiek van synergisme te hebben.

EU of nationale aanpak en methodiek ontwikkeling

Een sterk Europees traject ter afstemming van de methodiek voor meenemen van synergisme in de risicobeoordeling is noodzakelijk, ongeacht of er wel of geen besluit komt te liggen tot versneld ingrijpen in de toelating. Onder de verordening hebben lidstaten de mogelijkheid om specifieke nationale elementen mee te nemen in de beoordeling die de andere zonale lidstaten vervolgens niet beïnvloeden. Omdat het onderwerp bijen geen nationaal specifiek element is, zullen overige zonale lidstaten, conform artikel 44 van de verordening, moeten participeren of notificeren wanneer Nederland versneld ingrijpt. Bestuurlijk gezien kan Nederland dus niet om de zonale lidstaten heen.

Er is op dit moment geen Europees richtsnoer voor synergisme. Het ontwikkelen en harmoniseren van een richtsnoer zal jaren op zich laten wachten, waardoor een tussenoplossing moeten worden gevonden in samenwerking met de overige lidstaten voor

thiacloprid en acetamiprid. Het is daarbij mogelijk dat synergisme altijd ad hoc per stof moet worden beoordeeld omdat er geen algemene richtsnoer kan worden gemaakt.

Het advies aan het College is om een tussenfase van 3 maanden in te lassen in het project omdat er onvoldoende inzage is rondom de algemene problematiek van synergisme en het ontbreken van een EU geharmoniseerde richtsnoer voor synergisme. Hierdoor is het noodzakelijk geacht om eerst het algehele kader voor synergisme vorm te geven voordat er een beslissing kan worden genomen over herbeoordelen van de middelen o.b.v. thiacloprid en acetamiprid. In deze tussenfase zullen de volgende activiteiten worden verricht:

1. waardering van de studies aangaande synergisme van thiacloprid en acetamiprid met fungicides om het risico concreter te maken
2. onderzoek naar overige synergisme interacties op bijen om het algemene problematiek rondom synergisme concreter te kunnen inschatten. Hierbij zal ook een kritische analyse van EFSA opinie van 2012 worden gemaakt (zie bijlage II voor hoofdstuk 6 - synergisme in EFSA opinie)
3. overleg met kennisinstituten over synergisme en risicobeoordeling om een Nederlands inhoudelijk standpunt voor de EU te realiseren
4. overleg met de RMS en co-RMS van acetamiprid en thiacloprid om synergisme in de EU stofbeoordelingen te integreren en harmoniseren.

Er zal een sterke focus zijn op het Europees brede aanpak betreffende het harmoniseren van de stofbeoordeling, de algemene synergisme problematiek en voor eventuele voorgestelde maatregelen. We zijn voornemens om een advies aan de staatssecretaris uit te brengen naar aanleiding van het afronden van fase I. In dit advies zal de focus op het Europese traject worden benadrukt.

Aan het eind van de voorgestelde tussenfase zal wederom een advies worden uitgebracht aan het College in de vergadering van december. Het College wordt geadviseerd de Staatssecretaris aangehaakt te houden en zo nodig in te schakelen. Ook hier zijn wij voornemens om een advies aan de staatssecretaris uit te brengen naar aanleiding van het afronden van de tussenfase. Dit advies zal in januari worden verstuurd.

Analyse alternatief vervolgtraject - Risk mitigation measures en wijziging WG.
Een alternatief vervolgtraject is dat in de tussenfase niet een Europees brede aanpak wordt genomen maar dat er direct naar een herbeoordeling wordt gegaan, waar het risico nationaal wordt ingeschat en dat er vervolgens met de aanvragers wordt overlegd of zij via een wijziging WG de voorgestelde risico beperkende maatregelen willen opnemen.

In de herbeoordeling van bijen 2011 is de optie aan toelatinghouders geboden om een wijziging van het WG in te dienen om de nieuwe risicobeperkende maatregelen versneld in het WG te implementeren. Echter, de versnelde herbeoordeling van thiacloprid en acetamiprid valt onder Verordening (EG) 1107/2009, dit in tegenstelling tot de herbeoordeling van 2011 die onder de wettelijke implementatie van richtlijn (EEG) 91/414 viel. Het verschil zit hem er met name in, dat onder Verordening (EG) 1107/2009 een wijziging op aanvraag van de toelatinghouder (artikel 33) een inhoudelijke wijziging van de toelating is en dus óók zonaal dient te worden afgestemd. Het voordeel van een aanvraag tot wijziging WG van de toelatinghouders is dat er dan formeel geen zienswijzeprocedure nodig is. De besluiten worden dan sneller geëffectueerd, en NGO's kunnen zich er eventueel in bezwaar/beroep tegen verzetten.

Bij een complex vraagstuk als synergisme kan het opleggen van risico beperkende maatregelen alleen worden gedaan indien er een herbeoordeling ligt van de middelen. Er zijn 2 risico beperkende maatregelen mogelijk;

1. het wordt verboden om het middel in combinatie met triazolen te gebruiken. Dit is de risicobeperkende maatregel die Frankrijk heeft opgelegd voor acetamiprid. Echter, bij

blootstelling via pollen kan nog steeds een combinatie triazolen en acetamiprid en thiacloprid optreden als in omliggende velden triazolen wordt gebruikt.
2. gebruik van thiacloprid en acetamiprid verbieden in bij-aantrekkelijke gewassen. Dit zou gedurende het hele seizoen kunnen of alleen tijdens de bloei; de tweede fase van het project zou dit kunnen uitwijzen.

Bayer Cropscience heeft semi-veldstudies aangeleverd voor de herbeoordeling van thiacloprid en het is niet aannemelijk dat zij de restricties zullen accepteren zonder een goed onderbouwde herbeoordeling. Nisso International heeft geen aanvullende studies geleverd, maar heeft wel studies met stoffen interacties uitstaan voor het dossier van de herbeoordeling van 2014.

Een nationaal vervolgtraject wordt in deze fase van het project niet wenselijk geacht, en wordt dus ontraden.

Bijlage I - Nieuwe data

Nieuwe gegevens

In juni 2013 heeft het Ctgb de toelatinghouders, deskundigen en NGO's uitgenodigd om voor de herbeoordeling van thiacloprid en acetamiprid alle relevante data met betrekking tot het risico voor bijen vóór 5 juli 2013 aan te leveren.
Hierop is reactie gekomen van de toelatinghouders van thiacloprid en acetamiprid, te weten Bayer CropScience en Nisso Chemical Europe. Verder is reactie gekomen van Denka Registrations, Greenpeace, PAN Europe, 5.1.2.e en 5.1.2.e Aanvullend hierop heeft het RIVM in opdracht van Ctgb een literatuursearch uitgevoerd om zeker te zijn dat alle relevante literatuur aanwezig is.

Er zijn verschillende typen studies en artikelen aangeleverd. Hiervan is een groot deel direct relevant voor de risicobeoordeling naar bijen. Deze gegevens zijn onder te verdelen in:

- acute toxiciteit voor honingbijen van thiacloprid en acetamiprid, al dan niet met gelijktijdige blootstelling aan andere bestrijdingsmiddelen (fungiciden of diergeneesmiddelen)
- acute toxiciteit voor honingbijen van metabolieten van thiacloprid
- acute toxiciteit voor andere bijen (angelloze bijensoort) van thiacloprid
- acute toxiciteit voor andere bijen (hommelsoort) van acetamiprid
- chronische toxiciteit voor honingbijen van thiacloprid, al dan niet met gelijktijdige blootstelling aan *Nosema ceranae*
- chronische toxiciteit voor honingbijen van een metaboliet van thiacloprid,
- chronische toxiciteit voor andere bijen (hommelsoort) van thiacloprid
- subletale effecten op honingbijen (bijvoorbeeld op het geheugen)
- metabolisme van acetamiprid in honingbijen
- semi-veldstudies en veldstudies naar de effecten op honingbijen van thiacloprid of acetamiprid, al dan niet met gelijktijdige blootstelling aan andere bestrijdingsmiddelen (fungiciden)
- toxiciteit voor andere arthropoden dan bijen (zowel terrestrische als aquatische arthropoden)
- toxiciteit voor andere organismen dan arthropoden
- algemene monitoring van honingbijen
- studies naar residuen van thiacloprid en/of acetamiprid in bijenvolken, bloemen en guttatievocht
- artikelen die te maken hebben met de risicobeoordelingsmethodiek (o.a. *time-to-effect*)
- commentaar op de Europese beoordeling van thiacloprid
- informatie over toelatingen in Nederland van bepaalde fungiciden of synergisten op gewassen waar ook thiacloprid en/of acetamiprid zijn toegelaten
- een risicobeoordeling van acetamiprid uit Frankrijk waaruit blijkt dat er restrictiezinnen nodig zijn om toepassing wanneer bijen actief zijn te voorkomen
- lijst bij-aantrekkelijke gewassen (opgesteld door o.a. Ctgb)

Er zijn ook artikelen binnengekomen die betrekking hebben op de algemene problematiek over de achteruitgang van de bijenstand.

- stukken uit kranten en populaire tijdschriften
- politieke informatie (antwoorden op kamervragen, DG Sanco)
- statements WUR, ECPA
- visie bijenhouderij en insectbestuiving
- informatie over gebruik van Varroa-bestrijdingsmiddelen door imkers

Analyse van nieuwe data

Het Ctgb heeft een eerste screening uitgevoerd van de geleverde gegevens. Het grootste deel van de nu beschikbare gegevens is niet meegenomen in de Europese stofbeoordeling

van thiacloprid en acetamiprid. De EU stofbeoordeling is nu gebaseerd op enkele acute toxiciteitstudies en een enkele semi-veldstudie.

Uit de screening blijkt dat synergisme kan optreden tussen thiacloprid en acetamiprid met fungiciden en met piperonyl-butoxide (PBO), waarbij de toxiciteit van thiacloprid en acetamiprid met een factor 10 - 1000 kan worden verhoogd. Bayer Cropscience heeft semi-veld studies aangeleverd voor thiacloprid, waar synergisme niet lijkt op te treden tussen de stoffen. Nisso Chemical Europe heeft geen aanvullende studies aangeleverd aangaande synergisme voor acetamiprid.

Nieuwe acute studies
De acute toxiciteit van thiacloprid en acetamiprid alleen is juist vastgesteld in de Europese beoordeling. De stoffen zijn beide niet bijzonder toxisch voor honingbijen (LD50 tussen 1 en 100 µg a.s./bij). Echter, de acute toxiciteit voor honingbijen wordt in laboratoriumsetting duidelijk verhoogd bij (vrijwel) gelijktijdige blootstelling aan een bepaald type fungiciden en met PBO.

Nieuwe (semi-)veldstudies
De synergistische effecten met thiacloprid zijn ook onderzocht in meer realistische setting (semi-veldstudies). Hieruit blijkt geen verhoogde toxiciteit van thiacloprid met deze fungiciden. Voor acetamiprid zijn geen veldstudies aangaande synergisme aangeleverd.

Overige informatie
In een zeer summier beschreven aged residue proef bleek de verhoogde toxiciteit van acetamiprid in combinatie met triflumizole niet.
De overige geleverde informatie gaat onder andere in op metabolieten, chronische toxiciteit in het laboratorium, (semi-)veldtoxiciteit van de individuele stoffen, generieke monitoring en risicobeoordelingsmethodiek (inclusief mogelijke interactie met parasieten). Deze informatie geeft geen directe aanleiding tot herbeoordeling van thiacloprid en acetamiprid (een jaar voorafgaand aan de herregistratie). Deze gegevens zullen uiteraard wel besproken worden in de tweede fase van de herbeoordeling. Waar relevant worden ze meegenomen in de risicobeoordeling en indien nodig worden de huidige toelatingen aangepast.

Referentielijst:
Onderstaande referentielijst is nog niet volledig voor acetamiprid omdat het samenvat- en evalueerwerk in week 34 zal worden aangeleverd door RIVM.
Alle studies zijn opgeslagen in DMS:
http://intranet.ctgb.nl/openims/openims.php?mode=dms&currentfolder=(d1b73ca3374066cdf014e0876f8c48e4)20bc6f356219b947164ecee3bddbbc63

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Dág vlinders dàg bijtjes. Jop de Vrieze, NRC Handelsblad, Bijlage Wetenschap, 29-08-2009

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Rol van pesticiden ten opzichte van ziekte(verwekker)s: zie sheet 9 (imkers) en 10 (labs) van DG SANCO presentatie (Laddomada 2013)

Statement WU over pesticiden en bijen (5.1.2.e 2011)

Dubbele risicostudie door de PD in opdracht van het Ct(g)b over het voorkomen van sterfte van bijen en hommels (1997)

Overig

Ctgb lijst met bij-aantrekkelijke gewassen

Risicobeoordeling van Frankrijk voor Supreme 20 SW obv acetamiprid . Note: is hetzelfde middel als de NLse toegelaten middel Gazelle.

Risicobeoordeling voor bijen met betrekking tot de NLse toelatingen door Bayer.

Notitie van 5.1.2.e over synergisme en risicobeoordeling van de toegelaten middelen in Nederland.

Notitie PAN Europe aangaande studies in DAR van acetamiprid.

DAR thiacloprid, volume 3 - aangaande risicobeoordeling milieu

6 CHAPTER 6: HOW TO TAKE ACCOUNT OF CUMULATIVE AND SYNERGISTIC EFFECTS

6.1 Summary

Pesticides containing a number of active ingredients are frequently applied sequentially or as mixtures such as tank mixes, and there is a consensus in the field of mixture toxicology that the customary chemical-by-chemical approach to risk assessment is too simplistic. This chapter aims to review the evidence on cumulative and synergistic effects of pesticide mixtures in bees and to develop recommendations for risk assessment purposes.

In terms of cumulative risk assessment, there is evidence that concentration addition is a conservative method and in previous reviews, the estimated toxicity using this approach was concluded to be more conservative than that predicted by independent action. Generally, at sub-lethal doses, exposure concentration addition has been observed more often than synergistic or antagonistic effects for mixtures of pesticides with a common mode of action and independent action (response addition) has been observed for compounds with a different mode of action. In some cases synergistic and antagonistic effects have also been observed and can involve two types of interaction: toxicokinetic and toxicodynamic interactions. Toxicokinetic interactions at the level of the absorption, distribution, metabolism and excretion can result in a decrease (synergistic) or an increase (antagonistic) in metabolism or overall elimination of the compound and may affect toxicodynamics. Toxicodynamic interactions can result in increase (synergistic) or decrease (antagonistic) in toxicity.

In order to develop methodologies to take into account cumulative and synergistic effects of pesticides in bees, the toxicokinetic and toxicodynamic aspects of pesticide mixtures in bees was reviewed. Honey bees and hymenoptera are known to have a specific metabolic profile with the lowest number of copies of detoxification enzymes within the insect kingdom i.e cytochrome P-450, glutathione-S-transferases and carboxyesterases. A number of studies have shown synergistic effects of pesticides and active substances applied in hives as medicinal treatments against *Varroa* mites in honey bees, for which toxicokinetic interactions were most commonly involved. The mechanisms of such interactions involved inhibition or induction of either detoxification enzymes (cytochrome P-450) or transporters which then enhance the toxicity of the mixture and decrease the LD50. There is also a growing body of evidence that there may also be interaction between pesticides and honey bee disease (fungi, bacteria and viruses).

Currently, full dose responses for synergistic effects between potential inhibitors and different classes of pesticides are rarely available for either lethal effects or sub-lethal effects in bees so that predictions of the magnitude of these interactions at realistic exposure levels cannot be performed.

In the case of synergism which can be predicted based on the mode of action of the chemical classes involved (e.g. azole fungicides and insecticides), and in the absence of existing data on toxicity of the mixture, it is recommended to design full dose-response studies in adult bees and larvae for mixtures of potential synergists. These should take into account the dose dependency of the synergy, the magnitude of the interaction at concentrations of environmental relevance as well as both the maximum potentiating factor of the synergist and the concentrations for which no potentiating factor occur in the dose response curve. Such statistically sound dose response data will provide a basis to derive benchmark doses and their limit as suggested by EFSA's scientific committee. This flexible approach would allow quantitative protection goals to be achieved (e.g. specific effect size for lethality or for a sub-lethal effect depending on the protection goal and the aim of the risk assessment). Further work is also required to identify the molecular basis of interactions between environmentally realistic exposure to pesticides and the range of honey bee diseases (fungi, bacteria and viruses) to determine whether and how these may be included in risk assessment.

6.2 Introduction

In the environmental risk assessment of plant protection products, normally only the active ingredient or the formulation/product is taken into account. When the formulation contains more than one compound toxicity tests with the formulation have to be made available for the dossier (often only available for the most sensitive test species). In that case it is possible to compare the outcomes and to assess whether one of the compounds in the formulation behaves like a synergist.

When a compound or formulation/product is applied more than once in the growing season, the number of applications is taken into account in the registration process. However, only when the label of the formulation/product mentions that the formulation/product is used in a tank mix is the overall toxicity of the tank mix calculated on the basis of the dose (concentration) additivity; the tank mix as such is never tested. Note that in the tank mix not only could different compounds be mixed, but also additives, like for instance stickers and synergists, that will enhance the performance of the mixture.

The use of other pesticides in the same crop/field in the growing season or on neighbouring crops/fields are not taken into account in the environmental risk assessment. Organisms living in or close to such a field can be exposed many times (sometimes between 10 and 20 times) to one or more compounds (up to 4 is not an exception, see Chapter 2). In addition other compounds may be encountered in one of the environmental compartments. For instance, in the surface water, because neighbouring farmers or farmers upstream have used other compounds at the same time or during the same week. Vapour drift can also occur over relatively long distances from the source where a plant protection product was used.

6.3 Type of mixtures

6.3.1 Tank mixing

One of the few studies dealing with the contents of tank mixes was published by Fryday, Thompson and Garthwaite in 2011. The results of this study are summarized for 4 different crop types (e.g. arable crops, vegetable crops, orchards and soft fruit) in Table 6.1.

Table 6.1: Summary of applications for four different groups of crops

Crop type	Compounds in mixture	Mean a.i. per application	Mean a.i. in mixture	Unique combinations	% of total treated area	Year
Arable	2-9	3.26	6.15	5992	66	2008
Vegetable	2-7	1.49	2.81	1519	53	2007
Orchards	2-8	1.64	3.09	1099	60	2008
Soft fruit	2-6	1.58	3.24	891	46	2006

This shows that applications to 66% of the treated arable crop area contain an average of 6.15 compounds per application. For the other three crop types approximately 50% of the treated area is on average treated with three different compounds per application. These data show that the use of tank mixes in agriculture is a common phenomenon.

6.3.2 Sequential exposure

Wildlife may not just be exposed to mixtures of compounds due to tank mixes. There is also the possibility that wildlife will be exposed to mixtures of compounds following sequential applications to crops or as they move between treated fields.
Research carried out in the Netherlands gives indications that wildlife living in or nearby a particular crop can/will be exposed either many times to the same compound and/or to many different compounds within one growing season (Spruijt et al., 2010; Luttik et al., in prep.). In Table 6.2 a

number of standard crop scenarios and in Table 6.3 a number of realistic worst case scenarios are presented.

Table 6.2: Summary of applications within one growing season for 11 crops (taken from Spruijt et al., 2010)

Crop (Standard scenario)	Number of times compound has been applied (n)	Fungicides	Insecticides	Herbicides	Others
Strawberries 2006	26	12	4	8	2
Asparagus 2006	16	6	5	5	-
Consumption potatoes 2008	23	18	2	3	-
Hyacinths 2008	29	8	11	10	-
Narcissus 2008	15	9	-	6	-
Leeks 2008	24	9	8	7	-
Sugar beet 2008	10	1	-	9	-
Tulips 2008	33	8	10	15	-
Winter carrots 2006	13	5	4	4	-
Winter wheat 2006	9	4	1	4	-
Seed onions 2008	26	14	3	8	1

Table 6.3: Summary of applications within one growing season for 6 crops (taken from Luttik et al., in prep)

Realistic worst case crop scenarios	Number of times compound has been applied (n)	Fungicides n + number of compounds	Insecticides n + number of compounds	Herbicides n + number of compounds	Others n + number of compounds
Fruit 1	52	35 (9)	11 (8)	6 (5)	-
Fruit 2	48	32 (10)	9 (8)	7 (4)	-
Tuber 1	21	11 (4)	7 (2)	3 (3)	-
Flower 1	36	8 (3)	14 (3)	14 (5)	-
Flower 2	82	40 (5)	29 (3)	18 (5)	-
Flower 3	52	22 (5)	15 (6)	15 (3)	-

In Figure 6.1 the sequential use of plant protection products is shown for the flower 2 realistic worst case scenario (according to Luttik et al., in prep). All applications are applied in a period of 26 weeks.

This information clearly shows that wildlife can be exposed to a multitude of compounds several weeks in succession.

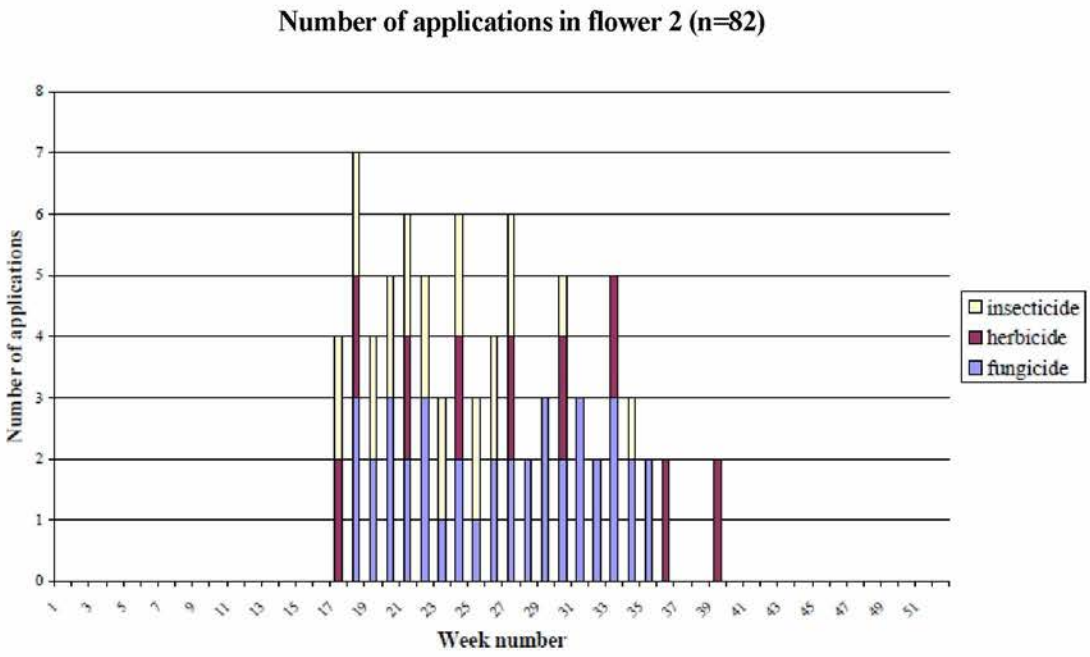

Figure 6.1: Sequential use of plant protection products

6.4 State of the art of mixture toxicology

There is a consensus in the field of mixture toxicology that the customary chemical-by-chemical approach to risk assessment might be too simplistic. There is a real possibility of underestimating the risk of chemicals to the environment. In binary and multiple mixtures of pesticides, most often concentration addition (CA) has been observed at low dose of exposure for compounds with a common mode of action (MOA) or independent action (IA) (response addition) for compounds with a different MOA. In some cases, a response in between concentration addition and response addition has been noticed. Mixture effects cannot be ruled out, even when all compounds in a mixture with different MOA are present at their individual NOECs.

Synergistic or antagonistic effects are more uncommon (see paragraph below). Indeed, deviations from the predictive concentration addition model, indicative of synergisms or antagonisms, are comparatively rarer, relatively small and largely confined to mixtures with only a few compounds.

In principle, the toxicology of mixtures involves two potential types of interactions: toxicokinetic and toxicodynamic interactions.

Toxicokinetic interactions may cause deviations from additivity between the components of the mixture

1) Absorption and distribution: a substance (B) in a mixture may compete with the absorption of another substance (A) in the mixture or inhibit active transport or efflux pumps and affect the circulating levels of substance A (internal dose) in the body.

2) Metabolism and excretion: typical examples of such interactions include inhibition or induction of metabolising enzymes (such as cytochrome P-450) by chemical components of the mixture. Taking a binary mixture, with chemical A acting as a competitive inhibitor of the metabolism of chemical B, the elimination of chemical B might be slower (decrease in clearance, increase in half life of the chemical). Consequently, a change in elimination patterns may modify the patterns of the delivery of the dose of the chemical to the target organ and can potentially increase the toxicity of the mixture.

Toxicodynamic interactions involve interactions between the biological responses from exposure (internal dose) to the individual substances in the mixture. A typical example are interactions of chemical sharing similar biological targets (e.g., ligand-receptor interaction) or MOA such as triazole fungicides in mammals assessed by the PPR panel (EFSA, 2009a).

In a recent review for the European Commission (Kortenkamp et al., 2009), the use of the concentration addition model was proposed as the concept of mixture toxicity that is most relevant for hazard characterisation and ultimately can be integrated into the legislative process for risk management purposes. The use of the concentration addition has also been discussed by Verbruggen and van den Brink (2010). There are two reasons that make the use of this model concept attractive for policy makers. First, the model concept is generally more conservative than the concept of response addition. Nevertheless, the magnitude of the differences at low levels of exposure between the two models is usually small and hence, the outcome will not be overly conservative. A second reason for the use of concentration addition is that the model concept can make use of existing data such as a NOEC, EC10 or EC50's by applying the concept of toxic units (TUs).

The concept of TUs has been recently reviewed by the three non food committees of the European Commission (the Scientific Committee on Health and Environmental Risks (SCHER), the Scientific Committee on Emerging and Newly Identified Health Risks (SCENHIR), the Scientific Committee on Consumer Safety (SCCS)) which defined TUs as "the ratio between the concentration of a mixture component and its toxicological acute (e.g. LC50) or chronic (e.g. long-term NOEC) endpoint". In addition, the toxic unit of a mixture (TUm) has been defined as the sum of TUs of each individual chemical of that mixture. The committees also noted that the TUs concept only refers to a specific organism representative of a group of organisms ecologically or taxonomically relevant for the ecosystem (e.g. algae, daphnids and fish for the freshwater ecosystem) but not to the ecosystem as a whole (SCHER/SCENIHR/SCCS, 2011).

In practice, TUs can be used to quantify the toxicity of a mixture (assuming the dose/concentration addition principle) based on its composition. For instance, an acute lethal TUm of 10 would mean that a dilution of 10% of the mixture would produce 50% of lethality. If the slope of the concentration/effect curve is known, the TUm can be used to estimate the expected effect. The applicability of the TUs are currently-limited for the response addition model since it would require full dose-response relationships for all species and all compounds to be assessed and such detailed toxicological data is not usually available.

The application of TUs to environmental concentrations (EC) (predicted PECs, or measured, MECs) has been compared with the Hazard Quotient (HQ) approach used in human risk assessment. However, two major differences were noted: (1) HQ are then added into Hazard Index (HI) instead of TUm, (2) TUs refer to the ratio between exposure and a toxicological endpoint whereas HQ refer to the ratio of exposure to a Reference Value (RV) such as a Tolerable Daily Intake (TDI) derived using uncertainty factors from the toxicological endpoints (i.e. No Observed Adverse Effect Level (NOAEL), Benchmark Dose and its limit (BMD and BMDL) (SCHER/SCENIHR/SCCS, 2011; EFSA, 2009a).

Generally, the RV in ecotoxicology is the Predicted No Effect Concentration (PNEC), so that the sum of PEC/PNEC ratios could be assumed as comparable to HI since PNEC are derived by applying uncertainty factors to the toxicological endpoint in the most sensitive species which may be different depending on the chemical. Because of such species differences, PEC/PNEC for component of a complex mixture were concluded to be non homogeneous and cannot be added (SCHER/SCENIHR/SCCS, 2011).

Besides the toxic unit approach, other applications for the concentration addition concept can be used like the Toxic Equivalent Factor (TEF) approach, the Hazard Index (HI), the Point of Departure Index (PODI) and the Relative Potency Factors (RPF). These approaches are described in the EFSA/PPR

opinion on cumulative and synergistic risks from pesticides to human health (EFSA, 2008a; Kortenkamp et al., 2009).

A promising new development in the field of mixture toxicity is the modelling of the effects of sequential exposure instead of simultaneous exposure. A model that can be used for this purpose is the Threshold Damage Model of Ashauer et al. (2007). This model describes the cumulative (acute) toxicity of compounds that are not used simultaneously but sequentially, which is a common feature in agriculture. The use of such a model is currently limited, since the parameters describing toxicokinetics and toxicodynamics are only available for a few species.

Another important development is the application of species sensitivity distributions (SSD) in the field of mixture toxicity. This method (Posthuma et al., 2002), which is often referred to as multiple-substance potentially affected fraction (ms-PAF), calculates the percentile of species affected by the exposure to multiple substances at the same time (this is also applicable to concentration addition and response addition).

Finally, the concept of MOA is promising for mixture assessment in ecotoxicology, however, its applicability may differ from the MOA used in human risk assessment. Indeed, the three non-food committees of the EU further discussed the potential differences in the relevance of endpoints for human risk assessment versus ecological risk assessment. Ecotoxicological end-points are broader than endpoints used in human risk assessment since they relate to ecologically-relevant parameters (i.e mortality, fertility, reproductive capability). In contrast, in human RA, there are some effects such as molecular markers of carcinogenicity which are important for individuals of the population but would have negligible relevance in ecotoxicology. Importantly, in ecotoxicology, knowledge of the toxicological MOA on all the different types of species that may be present in an ecosystem is very much incomplete. This is well exemplified for pesticides with MOAs that are well characterised in target organisms but scarce for non-target organisms. In this case, pesticides may target a particular physiological or metabolic function that may not be common to all species in the ecosystem especially for species that are far taxonomically and for non-target organisms the effect of the chemical is likely or often assumed to be of the narcotic-type (baseline toxicity). For example, organophosphate and chlorinated insecticide toxicity in algae is "baseline", narcotic-type whereas triazines toxicity are a magnitude higher since they inhibit photosynthesis. Hence, "common MOA" in ecotoxicology may refer to broad end-point (reproduction impairment, population growth, mortality, etc.) (SCHER/SCENIHR/SCCS, 2011).

6.5 Examples of synergistic effects in ecotoxicology

Examples of synergistic effects of pesticides in invertebrates are presented below in aquatic and terrestrial organisms and finally in bees to give the reader a broad perspective of work conducted to date.

6.5.1 Examples of synergistic effects between pesticides in aquatic organisms

Both the WiGRAMP report (COT, 2002) and Verbruggen and van den Brink (2010) suggest that synergistic and antagonistic interactions are rarely observed but there are some exceptions (see Table 6.4).

Table 6.4: Examples of combinations resulting in synergism (from Verbruggen and van den Brink, 2010)

Compounds in mixture	Mode/Site of action	Species tested	Deviation from Concentration addition
Pirimicarb/monocrotophos	Similar	*Tilapia nilotica*	2.7-fold
Quinalphos/phenthoate	Similar	*Oreochromis*	10-fold

		mossambicus	
Malathion/dioxathion	Similar	*Salmo gairdneri*	8.2-fold
Carbaryl/phenthoate	Similar	*Channa punctatus*	2.2-fold
Atrazine/trichlorfon	Dissimilar	*Chironomus tentans*	3.8-fold
Atrazine/malathion	Dissimilar	*Chironomus tentans*	2.8-fold
Deltamethrin/carbaryl	Dissimilar	*Lymnaea acuminata*	20-fold
Anilazine/tri-allate	Dissimilar	*Chlorella fusca*	3.5-fold

Organophosphorus esters and carbamates

Laetz et al. (2009) assessed the combined effects of organophosphorus esters (diazinon, malathion, chlorpyrifos) and carbamates (carbaryl, carbofuran) on the Coho salmon and showed significant synergism through increased brain AChE inhibition following exposure to mixtures of organophosphates or organophosphates and carbamates resulting in some cases in death of the fish (chlorpyrifos + malathion and diazinion + malathion). The basis for synergism between these pesticides would be expected to be a toxicodynamic interaction at the target receptor (cholinesterase). In addition, organophosphates have been used in a targeted approach to break resistance associated with pyrethroids; in combination the organophosphates appear to inhibit P 450 metabolism of certain pyrethroids resulting in an increased toxicity through a toxicokinetic interaction (Ahmed, 2009).

Herbicides and insecticides

Mixtures of herbicides and insecticides can also act synergistically. The herbicide atrazine in binary mixtures with the insecticides chlorpyrifos, diazinon and methyl parathion showed synergism in the amphipod *Hyalella azteca* (Anderson and Lydy, 2002). The mechanism of action was proposed to be induction of the P450 by atrazine increasing the rate of conversion of the parent thion to their active oxon forms resulting in increased AChE inhibition.
The interaction of herbicides with insecticides has also been reported in gibberellin inhibitor plant growth regulators (e.g. flurprimidol, paclobutrazol and triexapac-ethyl) which have also been identified as P450 inhibitors (Ramoutar et al., 2010) and synergize the activity of pyrethroids in coleoptera. Therefore mixtures of herbicides which interact by inducing or inhibiting P450s with pesticides which are also activated or metabolized by these enzymes may result in synergism of toxicity.

EBI fungicides and insecticides

The EBI (ergosterol biosynthesis inhibiting) fungicides are widely reported to inhibit vertebrate and invertebrate P450s and include major classes such as the Demethylation Inhibitors (DMI)-fungicides (imidazoles, triazoles, piperazines, pyrimidines, pyridines) and the amines (piperidines, morpholines, spiroketalamines). The toxicity of these compounds has been extensively reviewed in Thompson (1996). More recently synergism between EBI fungicides and neonicotinoid insecticides (thiacloprid + propiconazole) has been reported (Iwasa et al., 2004) but this has not been demonstrated at field realistic rates following sequential applications (Schmuck et al., 2003).

Recently, the joint effects of chemical mixtures on the life-history traits of *Daphnia magna* Straus were investigated. For instance imidacloprid was tested together with thiacloprid and imidacloprid with nickel chloride. For the mixture exposure of imidacloprid and thiacloprid, a synergistic pattern was observed in sub-lethal doses (number of neonates produced), while for the body length the best fit was shown with the CA model. In the mixture exposure of imidacloprid and nickel, no deviation from the IA was observed for the neonate production data; for the body length parameter, a synergistic pattern was observed in low doses of the chemicals (Pavlaki et al., 2011).

Recently, Bjergager et al. (2011) investigated the magnitude of the synergism between the conazole fungicide prochloraz and the pyrethroid (esfenvalerate) at environmentally realistic concentrations on

zooplankton and phytoplankton at days 0, 1, 2, 4, 7, 14, 21, and 28 after pesticide application by comparing EC20-values estimated on the basis of concentration–response curves for days 2, 4, and 7. Hence, prochloraz was shown to enhance the toxicity of esfenvalerate four to six fold for copepods and three to sevenfold for cladocerans with an indication of stabilisation or the beginning of recovery between day 7 and day 14 and full recovery in some of the less affected populations of cladocerans, copepods, and chironomids after 28 days. Authors concluded that the occurrence of the synergistic interactions between prochloraz and esfenvalerate in the microcosms and at environmentally realistic concentrations implies that the synergistic interactions may also take place in invertebrate communities in natural ponds and ditches being exposed to azoles and pyrethroids via for example runoff or drift. Authors discussed ways to tackle the question of synergy between chemicals in environmental risk assessment and proposed two approaches:

1) Testing maximum potentiating factor of proposed synergists towards high risk chemicals such as pyrethroids, to determine the size of extra uncertainty factors to be added to the pesticides having the synergy and including more sensitive species in tests

2) Investigate within the dose response curve, the dose at which no potentiating factors occur between the compound tested and the synergists (Bjergager et al., 2011).

6.5.2 Cumulative and synergistic effects of pesticides in insects and invertebrates other than bees

Synergistic toxicity of pesticides has been measured in a number of terrestrial athropods and annelids and nematods. For example, the synergy of atrazine and organophosphate insecticides has been demonstrated in midges (*Chironomus tentans*) (Pape-Lindstrom and Lydy 1997). In the earthworm, *Eisenia fetida,* atrazine and cyanazine increased the toxicity of chlorpyrifos 7.9- and 2.2-fold and body residue analysis suggesting that the greater-than-additive response may be due to increased biotransformation to more toxic oxon metabolites (Lydy and Linck, 2003). Recently, statistically significant dose-dependent synergism was also shown in the nematode *C. elegans* ($P<0.01$) whereas concentration addition was measured on *E. fetida* after exposure to similarly acting neonicotinoid pesticides imidacloprid and thiacloprid. Authors highlighted that these results show that deviations from concentration addition can happen even with similar acting compounds, but that the nature of such deviations are species dependent and concluded that the concentration addition model may need to be used in a probabilistic context, rather than in its traditional deterministic manner (Gomez-Eyles et al., 2009).

Reproductive toxicity of 10 binary mixtures of five different pesticides from three classes of neurotoxic pesticides with the same MOA (neuroexcitation) but different molecular mechanisms were tested in binary mixtures with the nematode *Caenorhabditis elegans*. Both CA and IA were found to be valid models for prediction of the toxicity of 4 of the mixtures, however, evidence for interaction was found in the remaining six cases and could be explained by toxicokinetics-interaction i.e. production of a metabolically activated or a metabolically deactivated chemical and/or cases where the relative potencies of the two tested chemicals differed greatly. The authors concluded that detailed analysis of toxicokinetics and toxicodynamics can aid further understanding of interactions in mixtures (Svendsen et al., 2010).

Finally, synergistic effects of insecticides (bifenthrin, imidacloprid) on tawny mole cricket (*Scapteriscus vicinus Scudder*) adults and nymphs, have been shown by injecting 5 µg per insect of each compound either as single compound or a binary mixture. Bifenthrin and imidacloprid provided the fastest median mortality causing immediate knockdown of the insects when injected and LD_{50} values for bifentrin and imidacloprid increased by 3.8- and 8.8-fold respectively in adults and 1.5 and 19-fold in nymphs. The authors concluded that the combination of the sodium channel toxin (bifenthrin) and the synaptic toxin (imidacloprid) lead to the synergistic effects, which to the authors'

knowledge provides the first documented evidence of synergistic neurological ''potentiation'' (Kostromytska et al., 2010).

Natural conditions resulting from the interaction between "natural" and chemical (anthropogenic) stressors can have dramatic effects on environmental species and such effects were recently reviewed for more than 150 studies and included stressors including heat, cold, desiccation, oxygen depletion, pathogens and immunomodulatory factors combined with a variety of environmental pollutants. Overall, synergistic interactions between natural stressors and chemicals were reported in more than 50% of the available studies on these interactions. Antagonistic interactions were also detected, but in fewer cases (Holmstrup et al., 2010).

6.5.3 Cumulative and synergistic effects of pesticides and active substances applied in hives as medical treatments in bees

Bees have specific features in their genome and at the level of detoxification enzymes that influence the toxicokinetics of pesticides. These genetic and metabolic particularities in bees are briefly summarised below.

6.5.3.1 Metabolic and toxicokinetic particularities in bees

Recent sequencing of the honey bee *(Apis mellifera)* genome has revealed that it lacks DNA methylation genes, major transposon families, genes for innate immunity and detoxification enzymes, cuticle-forming proteins and gustatory receptors. In contrast, *Apis mellifera* genome has more genes for odorant receptors, and novel genes for nectar and pollen use consistent with its ecology and social organisation (Johnson, 2008).

In terms of detoxification enzymes, honey bees possess only about half as many glutathione- S-transferases (GSTs), cytochrome P450 monooxygenases (CYP) and carboxyl/cholinesterases (CCEs) compared to other insects. This includes 10-fold or greater shortfalls in the numbers of Delta and Epsilon GSTs and CYP4 P450s, members of which clades have been recurrently associated with insecticide resistance in other species. It has been hypothesised that such shortfalls may contribute to the sensitivity of the honey bee to insecticides. On the other hand there are some recent radiations in CYP isoforms (CYP6, CYP9) and certain CCE clades in *A. mellifera* that could be associated with the evolution of the hormonal and chemosensory processes underpinning its highly organized eusociality (Johnson, 2008).

Regarding CYP genes, honey bees have one of the lowest number of isoforms of any inveterbrate sequenced to date (46 sequences), with the exception of fleas (*Pediculus humanus humanus*) (37 sequences) compared to 89 in *Drosophila melanogaster* and 111 in *Anopheles gambiae.* In comparison with other hymenoptera, the sequencing of the genome of the parasitic wasp (*Nasonia vitripennis),* which is haplodiploid as is A. *mellifera, has* revealed that this solitary parasitoid has twice as many CYPs as the honey bee with 92 CYP isoforms encoded in its genome. The difference between these two insects is most striking in the CYP4 clan, a poorly characterized group of CYP since *N. vitripennis* codes for 29 CYP4 P450s while *A. mellifera* includes only four. The CYP3 clan, which is associated with xenobiotic metabolism in other insects, is also reduced in *A. mellifera* compared to *N. vitripennis*. This pattern provides evidence that the well-regulated nest environment and diet of A *mellifera* constitute the principal factors in the low number of encoded CYP genes (Claudianos et al., 2006). From an evolutionary perspective, eusociality in bees and the high level of nest homeostasis insulate the queen from exposure to toxins making CYP-mediated detoxification less critical compared with other insects. Additionally, bees have a long evolutionary history of consuming processed nectar and bee bread resulting in a specialised exposure to phytochemicals and a low exposure to other environmental toxins, reducing the need for detoxicative enzymes (Claudianos et al., 2006; Johnson, 2008). These particular life style features of bees may explain the evolution of a lower number of CYP isoforms and the expression of specific CYP isoforms compared with other insects.

As an example, the CYP6AS subfamily (isoforms 1-10), which is apparently unique to hymenopterans is relatively dominant in honey bees. A number of CYP6AS isoforms (CYP6AS1, CYP6AS3, CYP6AS4) have been shown to play a role in processing phytochemicals encountered by bees in diet from concentrated processed nectar and bee bread. Indeed, quercetin, a compound present in honey, is a substrate for CYP6AS1, CYP6AS3 and CYP6AS4 isoforms and induces transcription of all three genes (Mao et al., 2011). Other isoforms have also been shown to be induced by honey extracts (CYP9Q2/CYP9Q3) suggesting that diet-derived phytochemicals may be natural substrates and may influence the ability of bees to detoxify pesticides (Johnson et al., 2009; Mao et al., 2011).

6.5.3.2 Cumulative and Synergistic effects of pesticides in honey bees

Prochloraz and deltamethrin's synergistic interactions were investigated in summer and winter bees. Individual compounds were used at sub-lethal doses that did not induce any significant mortality. Bees were treated with different doses of deltamethrin, either alone or in combination with prochloraz, at the constant field rate of 25 g/ha. In summer bees, the combination of prochloraz and deltamethrin at 125 mg/ha triggered a synergy that produced approx. 63 % mortality (corrected) after 24 h and at 62.5 mg/ha, deltamethrin's synergy with prochloraz induced about 32.5 % mortality (corrected) after 24 h. and the field rate of 31.2 mg/ha was the lowest dose at which deltamethrin acted in synergy with prochloraz in summer bees. In winter bees, no synergy occurred between prochloraz and deltamethrin at doses of 125 and 250 mg/ha and synergy was only observed at a deltamethrin dose of 500 mg/ha and produced 48% mortality (corrected) after 24 h. Overall, summer bees were shown to be approximately eightfold more susceptible than winter bees to the synergistic action of prochloraz and deltamethrin (Meled et al., 1998).

Pilling et al. (1995) published in vivo studies on [14C]-l-cyhalothrin metabolism showing that prochloraz inhibits pyrethroid metabolism in honey bees. Thus, Meled et al (1998) concluded that higher CYP- metabolism in winter bees compared with summer bees would support the argument that prochloraz inhibits pyrethroid metabolism. Conversely, a lower oxidative metabolism of pyrethroids in winter bees than in summer bees would be consistent with the hypothesis that deltamethrin is distributed to the tissues more readily and is more toxic.

Thompson and Wilkins (2003) have assessed the synergy and repellency of combinations of pyrethroids /fungicide mixtures in bees using acute toxicity tests (LD_{50}) and consumption of sucrose respectively. Two pyrethroids (alpha-cypermethrin and lambda-cyhalothrin) and 8 fungicides (iprodione and thiophanate-methyl, carbendazim, prochloraz, chlorthalonil, flusilazole, difenconazole, propiconazole, tebuconazole) and their realistic combination were tested. Overall, six and three of the eight funcigides increased the toxicity of lambda-cyhalothrin and cypermethrin respectively, with a maximum decrease in LD_{50} and increase risk of 6.7 and 2.2 fold for lambda-cyhalothrin- prochloraz and alpha-cypermethrin –prochloraz.

Johnson et al. (2006) examined the effects of three compounds inhibiting CYP activities (piperonyl butoxide, PBO), carboxylesterases (COEs): S,S,Stributylphosphorotrithioate (DEF) and glutatione-s-transferases (GSTs) (diethyl maleate, DEM) on the toxicity of these pyrethroids cyfluthrin, lambda-cyhalothrin or tau-fluvalinate (table 1). Inhibition of P450s with PBO significantly enhanced the toxicity of all three pyrethroids tested, while inhibition of COEs with DEF significantly enhanced the toxicity of cyfluthrin and tau-fluvalinate. One hour after treatment with cyfluthrin, lambda-cyhalothrin or tau-fluvalinate, honey bees displayed ataxia or hyperactivity, depending on the dose, as expected for poisoning caused by pyrethroids. Across all treatments, toxicity of the three pyrethroids to adult bees varied by almost four orders of magnitude, with the greatest toxicity exhibited by cyhalothrin synergised with PBO (LD50 = 1.3 ng per bee) and with the lowest toxicity exhibited by tau-fluvalinate without inhibitor treatment (LD50 = 9450 ng per bee). The rank order of the toxicities of the three pyrethroids to honey bees was cyfluthrin (LD50 = 68 ng per bee) >cyhalothrin (LD50 - 103 ng per bee) »tau-fluvalinate (LD50 = 9450 ng per bee). In quantitative terms, toxicity of the three pyrethroids to bees was greatly synergised by the CYP inhibitor PBO whereas little synergism was observed for

the glutatione-s-transferases. Cyfluthrin which was relatively more toxic without enzyme inhibitors showed the least synergism (30-fold). Such results suggest that metabolic detoxification reactions, especially those mediated by CYP isoforms contribute significantly to honey bee tolerance to pyrethroid insecticides.

In terms of CYP inhibition, complex effects on the pyrethroid bifenthrin have been shown on CYP transcripts from honey bee mid-gut using RT-PCR with an induction of CYP9Q1 and CYP9Q2 and a repression of CYP9Q3 transcripts (Mao et al., 2011).

Laboratory bioassays were conducted to determine the contact honey bee toxicity of nitro and cyano substituted neonicotinoid insecticides applied to the dorsum of the honey bee thorax. Nitro-substituted compounds were the most toxic with LD50 values of 18 ng/bee for imidacloprid, 22 ng for clothianidin, 30 ng for thiamethoxam, 75 ng for dinotefuran and 138 ng for nitenpyram whereas cyano-substituted neonicotinoids exhibited a much lower toxicity with LD50 values for acetamiprid and thiacloprid of 7.1 and 14.6 mg/bee, respectively. CYP and COE inhibitors PFO and DEF, and DMI fungicides (triflumizole, propiconazole) were applied to anesthetized bees 1 h prior to the insecticide application at the level of 10 µg per bee. CYP and COE inhibitors and DMI fungicides increased honey bee toxicity of acetamiprid 6.0-, 244- and 105-fold and thiacloprid 154-, 1141- and 559-fold, respectively, but had a minimal effect on imidacloprid (1.70,1.85 and 1.52-fold,respectively) whereas GST inhibitors had no effects on toxicity compared with controls. In contrast, Acetamiprid metabolites, N-demethyl acetamiprid, 6-chloro-3-pyridylmethanol and 6-chloro-nicotinic acid when applied topically, produced no mortality at 50 mg/bee (Iwasa et al., 2004).

Recently, experiments were conducted at FERA (Thompson, personal communication) to investigate the dose dependency of the synergy between thiamethoxam and propiconazole in honey bees measured by changes in the LD_{50} of contact doses (estimated exposure level =0.224 µg/bee). The magnitude of the interaction at estimated exposure level was 1.5-fold (see Figure 6.2).

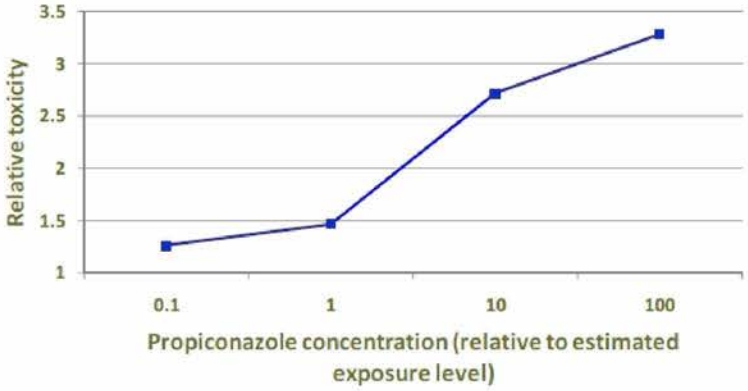

Figure 6.2: Increase in toxicity of thiamethoxam contact dose resulting from the co-application of propiconazole at differing doses (1= estimated exposure level = 0.224 ug/bee)

It is worth noting that in a number of papers on synergistic effects in bees, show synergists are several to 10 times above estimated environmental exposure in bees so that the magnitude of the interaction is an overestimation of the magnitude of the interaction that may be observed at an estimated level of exposure of synergists. Such data is not available for sub-lethal effects.

6.5.3.3 Synergistic effects between miticides applied to bee hives

Recently, Johnson et al. (2009) investigated the synergistic effects of the two miticides coumaphos and Tau-fluvinate by injecting the mixture in the thorax of three- to four-day-old bees using a microliter syringe mounted on a Hamilton PB-600 repeating dispenser. Pretreatment with CYP and COEs inhibitors exhibited a synergistic interaction with coumaphos, enhancing toxicity 2.8-fold and 4.0-fold, respectively whereas GST inhibitors did not have any influence. Synergism of tau-fluvalinate was also observed with coumaphos pre-treatment, with its toxicity exhibiting 2.1-fold synergism in the presence of as little as 0.3 µg coumaphos. Tau-fluvalinate toxicity increased in a dose-dependent manner with coumaphos as a pre-treatment increased: 2.5-, 4.4-, and 32.1-fold with 1 µg, 3 |µg, and 10 µg coumaphos respectively. The rationale for such interaction has been discussed previously and two important aspects would need to be considered: lipophilicity and competitive antagonism of the CYP enzyme. Both compounds are lipophilic and are absorbed by the wax component of the hive and can be persistent with the potential to build up over repeated treatments. Molecular modelling of the CYP9Q proteins and docking of tau-fluvalinate and coumaphos has recently shown that both miticide forms compete for the CYP isoforms resulting in competitive inhibition and decreased elimination of the compounds (Mao et al., 2011).

Recent data on P-glycoprotein inhibition and pesticide, miticide toxicity

P-glycoprotein or multiple drug resistance (MDR) transporters in bees have been recently shown to play a role in the synergistic effects of pesticides. Hawthorne and Dively (2011) showed that pre-treatment with a P-glycoprotein inhibitor (using the anti-hypertension drug verapamil) or the in-hive antibiotic (oxytetracycline) fed to bees significantly increased the toxicity (% mortality) of three neonicotinoid insecticides (imidacloprid, acetamiprid and thiacloprid insecticides) and two acaricides (coumaphos and t-fluvalinate). Increased mortality at higher concentrations and at the later end point (48 h) was observed for thiacloprid, and at 48 h for imidacloprid. For comparison with the verapamil synergism, mean bee mortality treated with 2 ug/ul coumaphos increased from 7% (n= 4 cages) to 51% (n= 4 cages) following feeding of OTC (1.4 mM), a significant but smaller increase than that caused by verapamil. OTC feeding increased the mortality of bees treated with 3 ug/ul t-fluvalinate from 5.6% (n= 10 cages) to 39% (n= 8 cages). The authors concluded in this preliminary study that all 5 compounds are substrates of one or more MDR transporters.

Overall, synergistic interactions in bees have been shown to result from toxicokinetic interactions at the level of metabolism either through the inhibition of a CYP or a transporter which then have toxicodynamic consequences enhancing the toxicity of the mixture/decreasing the LD50. However, full dose responses of such synergistic effects from potential inhibitors and different classes of pesticides are not available so that predictions of the magnitude of these interactions cannot be quantified in bees.

6.5.3.4 Synergistic effects between disease, malnutrition and pesticide toxicity in honey bees

Diseases and bee health

A number of bee diseases may result in adverse effects on bee health (Genersch et al., 2010), causing colony weakening or colony collapses. The most important diseases that occur regularly in Europe often spread from infestations with the *Varroa* mite, secondary infections with viruses (like Acute bee paralysis virus, Chronic bee paralysis virus, Deformed wing virus, Kashmir bee virus, Israeli acute paralysis virus) or infestations with the fungus *Nosema apis* or *Nosema ceranae* and other diseases such as European or American foulbrood. Such *Varroa* mites, *Nosema* spores and viruses are present in almost all colonies and may have severe impacts on colonies. Additionally, a number of factors influence the potential outbreak of clinical symptoms and are subject to great variation.

In semi-field or field trials with honey bee colonies, it is not possible to get comparable infestation rates with e.g. *Nosema* and *Varroa* or Viruses as it is in the laboratory. Indeed, *varroa* has not yet been

grown *in vitro* and achieving a standardised infection rate of bees and brood is difficult. Often, virus loads are detected in colonies without any clinical symptoms and factors leading to clinical symptoms are not yet fully understood. Infection with *Nosema* spores are difficult to handle and a controlled infection is only possible in the laboratory but still with some difficulties. Variability in response to *Nosema* in bees is very large. For example, during a *Nosema apis* infection in *Apis mellifera* with several different spore doses, it was not possible to establish a discernible relationship between bee longevity and spore dose in two races of European bee (Malone and Stefanovic, 1999). Infections of *N. ceranae* appear to have different effects on colonies in different geographical regions. Finally, seasonal variations and gross colony level symptoms (Fries, 2010) described for *N. apis* do not seem to be present in *N. ceranae*.

Recently, gene expression profiles from whole-genome microarrays between guts of bees from Colony Collapse Disorder (CCD) colonies originating on both the east and west coasts of the United States and guts of bees from healthy colonies sampled before the emergence of CCD, have been published. Indeed, the gut of bees acts as a primary interface between the honey bee and its environment (as a site of entry for pathogens and toxins). First, considerable variation in gene expression was associated with the geographical origin of the bees, although 65 transcripts were identified as potential markers for CCD status. Overall, no elevated expression of pesticide response genes was observed and genes involved in immune response showed no clear trend in expression pattern despite the increased prevalence of viruses and other pathogens in CCD colonies. However, unusual ribosomal RNA fragments, depicted through microarray analysis, were more abundant in the guts of CCD bees. The authors concluded that such fragments may be related to arrested translation as a possible consequence of picorna-like viral infection (including Deformed wing virus and Israeli acute paralysis virus) and that analysis of the RNA fragments' abundance and presence maybe a useful diagnostic marker for the CCD bee (Johnson et al., 2009).

Synergistic effects between diseases and pesticide toxicity in honey bees

There is growing evidence that interaction between honey bee disease and pesticide toxicity have synergistic effects on bee health. Alaux et al. (2010) investigated the interaction between the microsporidia *Nosema* and imidacloprid (0.7, 7 and 70 µg/kg) and showed increased sucrose consumption (i.e. increase in imidacloprid exposure) and consequently increased mortality. Overall, the combination of both agents caused, in the short-term, the highest individual mortality rates compared with controls. Haemocyte number and phenoloxidase activity, as markers of immunity, were quantified for both individual and social levels but neither markers were shown to be affected by treatment. In contrast, glucose oxidase activity used in honey bees for the sterilisation of the colony and brood food, was significantly decreased suggesting a synergistic interaction and in the long-term a potentially higher susceptibility of the colony to pathogens. Vidau et al (2011) showed synergistic effects on bee mortality between exposure to sub-lethal doses of fipronil or thiacloprid and *N. ceranae* infection compared with uninfected bees. Induction of phase I and phase II detoxification enzymes were also measured in mid-gut of the bees and only phase II (glutathione-s-transferases) were shown to be induced whereas phase I (cytochrome P-450 activity measured as 7-ethoxycoumarin-O-deethylase) was not. The authors also tested the effect of insecticide exposure on *Nosema* spore production. Fipronil and thiacloprid were shown to have opposite effects on spore production with a respective decrease and increase of about 33% and 40 %. The authors concluded that these results did not explain the mortality increase observed in the presence of insecticides and that further research is needed.

Recently, the infection dynamics of deformed wing virus (DWV), sacbrood virus (SBV), and black queen cell virus (BQCV) in adult bees, *Varroa* mite-infested pupae, s, and uninfested pupae, has been compared between bees treated with tau-fluvalinate and untreated control colonies. Initially, titres of DWV increased with the onset of the acaricide application and then slightly decreased progressively coinciding with the removal of the *Varroa* mite infestation and the authors concluded that the initial increase in DWV titres suggested a physiological effect of tau-fluvalinate on the host's susceptibility to viral infection. DWV titres in adult bees and uninfested pupae remained higher in treated colonies

than in untreated colonies. The titres of SBV and BQCV found had a variety of possible effects of tau-fluvinate (Locke et al., 2012).

One of the challenges raised by these studies is the difficulty to extrapolate to field conditions since comparable field studies have never been published and comparable infections under field conditions are very difficult, if not impossible, to achieve.

Malnutrition

The shortage of food may result in adverse effects on bee colonies. A lack of carbohydrates may result in weakening and consequently death of a bee colony whereas a lack of pollen will result in brood reduction, brood cannibalism, resulting in colony weakening and poor health status. Hence, good beekeeping practice would ensure that bees have access to good nutritional sources and that the landscape offers good foraging throughout the year. In normal conditions, also in agricultural areas, beekeepers are able to and will choose an appropriate location ensuring nectar and pollen flow. Beekeepers may feed sugar syrup to avoid carbohydrate starvation according to good beekeeping practice.

Bees fed high quality pollen appear less sensitive to pesticides than those fed with lower qualities or inadequate amounts of pollen or pollen substitute during development (Wahl and Ulm, 1983, von der Ohe and Janke, 2009). The amount of pollen collected by a colony could potentially be influenced by as many as 10 or more other variables (e.g. worker population size, number of larvae, surrounding vegetation, weather conditions etc.) (Keller et al., 2005). Nevertheless, the deprivation of protein status cannot be standardised for bee colonies. It is important to point out that policy makers should ensure that planting flowering crops or flower strips, maintaining and promoting biodiversity is of major importance to the health of honey bees and non-*Apis* bees and especially for maintaining non-*Apis* populations.

A recent study demonstrated, through an analysis of gene expression in bee midguts using northern blots, that honey, pollen and propolis induces detoxification enzymes in bees (CYP6AS), through the natural flavonoid quercetin and that mortality in bees exposed to the mycotoxin aflatoxin consuming sucrose or high-fructose corn was higher compared with bees exposed to aflatoxin and fed honey (Johnson et al., 2012).

Overall, there is a growing body of evidence that bees infected by parasites/pathogens may be more susceptible to chemical toxicity than healthy ones and that malnutrition also influences bee health. Active monitoring may be designed as "multifactorial studies" to investigate multiple factors putatively contributing to bee mortality including diseases. There is a wide variety of monitoring and surveillance systems for bee mortality and bee health which have been recently reviewed by an EFSA working group (Hendrikx et al., 2009).

6.6 Cumulative and synergistic effects

6.6.1 How to calculate concentration and response addition

Concentration addition (CA)

This approach is used where chemicals have the same site of action (simple similar joint action) but do not affect the biological activity of each other (no interaction). For this method the endpoint must be the same for each chemical.

$$\text{Total Toxicity} = (C_a/T_a + (C_b/T_b) + \ldots\ldots + (C_n/T_n) \qquad \text{Concentration addition (CA)}$$

Where C = concentration (or dose)
T = toxicity

Response addition (RA)

This approach is used where chemicals have different sites of action (independent joint action) but do not affect the biological activity of each other (no interaction). Here each component of the mixture acts on a different physiological or biological system but contributes to a common response.

This requires biological response (BR) expressed as % toxic effect for the assessed concentration from dose response curve for each constituent.

Total toxicity = $BR_1 + BR_2 + ... + BR_n$ Response addition (RA)

The disadvantage of this method is that it requires dose response data for all of the mixture constituents and species being assessed.

6.6.2 Comparisons of additive estimates with measured toxicity

There is evidence that CA is a conservative method for assessing the toxicity of mixtures (Kortenkamp et al., 2009; Verbruggen and van den Brink, 2010). In all cases analysed, the estimated toxicity using this approach was higher than that predicted by IA (Kortenkamp et al., 2009). When comparing estimates using CA, it has been estimated that the majority of estimates do not deviate by more than a factor of 2 (Deneer, 2000), 2.5 (Warne, 2003; Junghans et al., 2006) or 3 (Kortenkamp et al., 2009). There is also some evidence that this deviation is greatest for mixtures containing small numbers of chemicals and decreases as the complexity of the mixture increases. With respect to honey bees, the analyses performed by Deneer (2000), Warne (2003), Junghans et al. (2006), Kortenkamp et al. (2009) did not include toxicity data for honey bees. In addition, the mixture toxicity data presented in section 6.5 shows that in some cases magnitude of interactions can be higher than a factor of 3 although most studies have used synergist concentration which were often orders of magnitude above environmental concentrations. Finally, because of their specific toxicokinetic profile compared to other insects, synergistic interactions between pesticides in bees have a toxicokinetic basis (see section 6.5) and full dose responses of synergistic effects from potential inhibitors and different classes of pesticides at concentrations of environmental relevance.

Hence, applying a default uncertainty factor, such as an uncertainty factor of 2 to 3 to the threshold of toxicity for honey bees, would be premature until laboratory research has been undertaken with a number of mixtures of priority at relevant levels of exposure in adult bees and larvae.

6.6.3 Proposal for assessment of cumulative and/or synergistic effects

Synergism of pesticides in honey bees can either be predicted or assumed based on chemical class information (e.g. conazole (EBI) fungicides and insecticides) and knowledge of the mode of action/molecular targets of the individual pesticides in the mixtures. Therefore, in the absence of existing data on toxicity of the mixture, it may be necessary to conduct toxicological studies in adult bees and bee larvae using realistic application concentrations to determine the threshold of toxicity and the magnitude of the synergism. Finally, if the compound has the potential for bioaccumulation and repeat dose effects (Chapter 4), the risk assessment scheme proposed in Chapter 6 would require data for half life of the compound and its metabolites in adult bees and larvae. Such toxicokinetic information can provide a further understanding of the likelihood of pesticide synergism in honey bees.

The design of ecotoxicological studies for mixtures of potential synergists should take into account the toxicokinetics of the synergists (half life) and the dose dependency of the synergy. Consequently a full dose response can be generated to determine the magnitude of the interaction at concentrations of environmental relevance, and both the maximum potentiating factor of the synergist and the concentrations for which no potentiating factor may occur in the dose response curve.

Such statistically-sound dose response data will provide a basis to derive benchmark doses (BMD) and their limit (BMDL) as suggested by EFSA's Scientific Committee (EFSA, 2009b). Such benchmark doses are very rarely available in ecotoxicology and provide a flexible approach to reaching quantitative protection goals. For example, the dose response can consider a specific effect size for lethality (1%, 2% or 5%) or for a sub-lethal effect (described in Chapter 4-annexes) depending on the protection goal and the aim of the risk assessment. In this case, such a BMDL would be equivalent to an SSD (percentile of species affected by the exposure to multiple substances) (Posthuma et al., 2002) which is already applicable to concentration addition and response addition and can be applied to synergism.

Such a mechanistic approach has been proposed by the three non-food committees of the European Commission when dealing with ecotoxicological data of mixtures while acknowledging that very little mechanistic data is available in this field (SCHER/SCENIHR/SCCS, 2011). Overall, when sufficient studies have been conducted, a number of options would be available to the risk assessor and can be applied in future risk assessments i.e. to derive species-specific, chemical-specific, mixture-specific or class- specific adjustment factors using the full dose responses, the BMDs, BMDLs or SSDs and the magnitude of the interaction.

Bijlage III –Pesticides found in pollen trapped off honey bees returning to the nest.

Pettis JS et al. (2013) Crop Pollination Exposes Honey Bees to Pesticides Which Alters Their Susceptibility to the Gut Pathogen Nosema ceranae. PLoS ONE 8(7): e70182. doi:10.1371/journal.pone.0070182

Pesticide	Insecticide family	LD_{50} (ppm)[a]	Crops in which detected[c]	Detections	Quantity detected, mean±se (max) (ppb)	Relative risk (95% CI)
Fungicides						
Azoxystrobin		>1,562.5 [64]	Cr, Cu, Wa	10	60.3±25.6 (332)	0.75 (0.56, 1.02)
Captan		>78.13 [65]	Ap, Cr, Cu, Wa	9	976.9±734.4 (13,800)	0.59 (0.42, 0.81)†
Chlorothalonil		>1,414.06 [66]	Ap, Bl, Cr, Cu, Pu, Wa	17	4,491.2±2,130.7 (29,000)	2.31 (1.35, 3.94)†
Cyprodinil		>6,125 [67]	Ap	3	996.9±707.5 (12,700)	0.31 (0.15, 0.65)†
Difenoconazole		>781.25 [68]	Ap	3	171.4±119.4 (2,110)	0.31 (0.15, 0.65)†
Fenbuconazole		>2,282.65 [69]	Ap, Cr, Cu	10	227.3±89.2 (1,420)	0.33 (0.23, 0.48)†
Pyraclostrobin		573.44 [70]	Cr, Pu	4	2,787.1±1,890.1 (27,000)	2.85 (2.16, 3.75)†
Quintozene (PCNB)		>0.78 [71]	Cr	2	0.3±0.3 (4.7)	0.97 (0.59, 1.61)
THPI	Captan metabolite		Cr, Cu	3	832.1±531.8 (9,470)	0.42 (0.21, 0.82)†
Herbicides						
Carfentrazone ethyl		>217.97 [72]	Cr	1	0.1±0.08 (1.6)	1.05 (0.54, 2.05)
Pendimethalin		>388.28 [73]	Ap, Cr, Pu	5	5.1±3.7 (69.5)	1.47 (1.08, 1.99)†
Insecticides						
2,4 Dimethylphenyl formamide (DMPF)*	Amitraz (formamidine) metabolite		Bl, Cu, Pu, Wa	10	171.5±117.0 (2,060)	2.13 (1.56, 2.92)†
Acetamiprid	Neonicotinoid	55.47 [60]	Ap	3	59.1±32.2 (401)	0.31 (0.15, 0.65)†
Bifenthrin	Pyrethroid	0.11 [74]	Pu, Wa	3	6.6±3.8 (53.1)	2.08 (1.53, 2.83)†
Carbaryl	Carbamate	8.59 [75]	Ap, Cu, Wa	6	57.8±30.0 (403)	0.42 (0.27, 0.66)†
Chlorpyrifos	Organophosphate	0.86 [16]	Ap, Cr, Cu, Pu	7	3.1±1.1 (15.5)	0.89 (0.64, 1.23)
Coumaphos*	Organophosphate	35.94 [16]	Bl, Cr, Cu	6	2.2±1.0 (17.5)	0.62 (0.43, 0.91)†
Cyfluthrin	Pyrethroid	<0.31 [76]	Cr, Wa	2	0.6±0.4 (5.4)	1.31 (0.85, 2.02)
Cyhalothrin	Pyrethroid	0.30 [77]	Ap, Pu, Wa	7	14.6±7.9 (131)	0.94 (0.69, 1.29)
Cypermethrin	Pyrethroid	0.18–4.38 [78]	Cr	1	0.4±0.4 (6.9)	1.05 (0.54, 2.05)
Deltamethrin	Pyrethroid	0.39 [79]	Cr	1	4.5±4.5 (85.3)	1.05 (0.54, 2.04)
Diazinon	Organophosphate	1.72 [80]	Ap, Cr	3	1.4±1.0 (19.8)	0.56 (0.32, 0.97)†
Endosulfan I	Cyclodiene	54.69 [16]	Ap, Cr, Cu, Pu, Wa	8	1.5±0.7 (12.9)	1.60 (1.20, 2.14)†
Endosulfan II	Cyclodiene	54.69 [16]	Ap, Cr, Cu, Pu	6	0.8±0.3 (5.3)	1.41 (1.04, 1.91)†
Endosulfan sulfate	Endosulfan metabolite		Cr, Cu	4	0.3±0.2 (2.1)	0.79 (0.52, 1.19)
Esfenvalerate	Pyrethroid	0.13 [81]	Ap, Cr, Cu	7	16.9±12.0 (216)	0.51 (0.35, 0.75)†
Fluvalinate*	Pyrethroid	1.56 [82]	Bl, Cr, Cu, Pu, Wa	16	42.4±29.7 (570)	2.43 (1.49, 3.96)†
Heptachlor epoxide	Heptachlor[b] (cyclodiene) metabolite		Cr	1	0.6±0.6 (12)	1.05 (0.54, 2.04)
Imidacloprid	Neonicotinoid	0.23 [83]	Ap	3	2.8±2.0 (36.5)	0.31 (0.15, 0.65)†
Indoxacarb	Oxadiazine	1.41 [84]	Ap	2	0.5±0.5 (9)	0.28 (0.11, 0.73)†
Methidathion	Organophosphate	1.85 [85]	Cr	1	1.6±1.6 (31)	1.05 (0.54, 2.04)
Methomyl	Carbamate	<3.91 [86]	Wa	1	13.6±13.6 (259)	1.54 (0.91, 2.61)
Phosmet	Organophosphate	8.83 [85]	Ap, Cr, Cu	5	798.7±772.4 (14,700)	0.36 (0.21, 0.61)†
Pyrethrins	Pyrethroid	0.16 [16]	Cr	1	5.1±5.1 (97.4)	1.05 (0.54, 2.05)
Thiacloprid	Neonicotinoid	114.06 [60]	Ap	2	1.1±0.8 (12.4)	0.35 (0.15, 0.82)†
Control diets						
BRL	NA	NA	NA	NA	NA	0.58 (0.23, 1.48)
MegaBee	NA	NA	NA	NA	NA	0.74 (0.33, 1.67)

[a]We divided LD_{50} values given as *m*g/bee (g) by 0.128 (equivalent to multiplying by 7.8) to obtain ppm when necessary [85]. If multiple values have been published, we include only the smallest.

[b]Heptachlor has been banned for use on cranberries since 1978 [87], but can persist in the soil for extended periods of time.

[c]Ap = apple, Bl = blueberry, Cr = cranberry, Cu = cucumber, Pu = pumpkin, Wa = watermelon.

*Used by beekeepers within the hive for parasitic mite control.

†Relative risk different from 1 at the 95% confidence level.

NA indicates information that is not relevant to control diets.

doi:10.1371/journal.pone.0070182.t002

Bijlage IV. Aandachtspunten aangedragen door NGOs, deskundigen en industrie op 24 juli en 16 september 2013.

Verslag : Herbeoordeling acetamprid en thiacloprid aangaande risico voor bijen

Locatie : Ctgb

Vergaderdatum : 24 juli 2013
Aanwezig : 5.1.2.e (PAN Europe)
5.1.2.e (ETS Nederland BV)
5.1.2.e (Greenpeace)
5.1.2.e (Denka Registrations BV)
5.1.2.e (Ctgb)
5.1.2.e (Ctgb)
Afwezig : -

Volgende verg. :

Van : 5.1.2.e

Het Ctgb zal geen uitgebreid verslag maken, maar alleen aandachtspunten noteren.

Aandachtspunten:
Hieronder volgen de aandachtspunten die zijn geïdentificeerd tijdens het gesprek, die specifiek zijn voor de herbeoordeling thiacloprid en acetamiprid voor bijen

- 5.1.2.e Neem het voorzorgsprincipe als specifiek onderdeel mee in de herbeoordeling van thiacloprid en acetamiprid.
- 5.1.2.e In fase I waar WUR en RIVM als kennispartners optreden zouden meer NGOs/deskundigen moeten worden meegenomen.
- 5.1.2.e Benoem duidelijk het kader van beoordeling: wordt alleen de honingbij of worden alle wilde bijen meegenomen in de herbeoordeling.

Algemene aandachtspunten aangegeven door aanwezigen:

- 5.1.2.e geeft aan dat de eindconclusie voor toegelaten middelen door het Ctgb te stellig is naar zijn mening: dat een middel veilig is indien de voorschriften worden gevolgd. Hierbij is het onderscheid tussen toegelaten (veilig) en niet toegelaten (niet veilig) te zwart/wit.
- 5.1.2.e geeft aan dat de focus op bestrijdingsmiddelen in de bijenproblematiek buitenproportioneel is en de overige factoren (o.a. varroamijt en bloemendiversiteit) meer aandacht verdienen.

Contact personen voor herbeoordeling:

PAN Europe : 5.1.2.e en 5.1.2.e
Greenpeace : 5.1.2.e
Denka Registrations BV : 5.1.2.e en 5.1.2.e
ETS Nederland BV : 5.1.2.e
Ctgb : 5.1.2.e