Deriving threshold values for substances with a lower risk profile by applying TKTD models

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ToxicoKinetic ToxicoDynamic (TKTD) approaches are now becoming more widely used. This development was stimulated by the published EFSA opinion on TKTD modelling. A TKTD approach is based on time-independent parameter values of which the Threshold for Effects is the most important parameter value. In this report the TKTD approach was applied to compounds used in crop protection that are classified as 'limited' risk for aquatic organisms. Most low-risk compounds are biological by nature (being either organisms or being of biological origine) and therefore not suitable for chemical based approaches. The reason is that the equilibrium a TKTD model supposes does not exist between the biological pesticide and the target pest organism. Compounds with limited risk (compounds with a H412 or H413 hazard classification) were therefore selected from the EFSA pesticide database, which resulted in 14 synthetic chemical compounds. Effect thresholds could be estimated for a number of species/compound combinations. The database of two compounds included enough data to generate a Species Sensitivity Distribution (SSD). As a proof of concept an assessment based on effect thresholds is feasible and will generally lead to lower values than the standard risk assessment. Such an approach takes away most drawbacks from an LC50 or EC50 based approach and lower assessment factors may be used.

Keywords: low-risk, effect thresholds, TKTD models, risk assessment, pesticide directive, Water Framework Directive

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Beleidssamenvatting

Afleiden van drempelwaarden voor stoffen met een lager risicoprofiel met behulp van TKTD (Toxicokinetische-Toxicodynamische) modellen

Toxicokinetische-toxicodynamische (TKTD) modellen worden steeds meer toegepast in de risicobeoordeling voor gewasbeschermingsmiddelen. Bovendien zijn TKTD modellen voor de voorspelling van acute effecten op vis en invertebraten door EFSA (European Food Safety Authority) beoordeeld als 'fit-for-purpose'. TKTD modellen voor de voorspelling van chronische effecten op organismen worden nog niet als fit-for-purpose beschouwd en zijn in ontwikkeling. Het grote verschil tussen een standaard ecotoxicologische benadering en een TKTD model is dat bij de eerste aanpak de lethale of effect-concentratie voor 50% van de soorten wordt berekend, terwijl TKTD modellen tijdsonafhankelijke berekeningen kunnen uitvoeren en voor een oneindige blootstellingstijd een LC_0 berekenen die beschouwd kan worden als een drempelwaarde voor effecten. De vraag voor het uitgevoerde onderzoek was of TKTD modellen gebruikt kunnen worden voor het inschatten van de effecten van laag-risico stoffen en daarmee dus in de risicobeoordeling voor laag-risico stoffen gebruikt kunnen worden.

Om TKTD modellen te kalibreren en valideren, zijn effectconcentraties in de tijd nodig die gegenereerd worden in experimenten. Voor een dergelijke kalibratie / validatie zijn minimaal 3 datapunten in de tijd noodzakelijk.

Laag-risico stoffen zijn stoffen die in het kader van de verordening EC 1107/2009 voldoen aan de eisen voor toelating voor gebruik in de landbouw en tevens ook voldoen aan de criteria voor laag-risico stoffen. De meeste laag-risico stoffen zijn van biologische oorsprong. Dit kunnen natuurlijke stoffen zijn met een biologische oorsprong maar ook organismen die als actieve stof worden gebruikt, zoals micro-organismen (onder meer bacteriën, virussen, schimmels). Methodes die zijn gebaseerd op chemische middelen zoals de klassieke LC_{50} of EC_{50} gebaseerde evaluaties inclusief TKTD modellen zijn niet geschikt voor het gebruik in de risicobeoordeling van middelen die gebaseerd zijn op de biologische actieve middelen zoals microbiële middelen en feromonen. Deze biologische middelen infecteren het plaagorganisme, waarna ze zich vermeerderen in het plaagorganisme. Methodes die zijn gebaseerd op chemische stoffen veronderstellen een geleidelijke opname en mate van eliminatie van de actieve stof door het plaagorganisme, die uiteindelijk leidt tot een evenwicht tussen de blootstelling in omgeving en in het plaagorganisme. Door de groei van de biologisch actieve stof in het plaagorganisme wordt een dergelijk evenwicht per definitie nooit bereikt. Deze middelen vereisen een heel ander type van risico-evaluatie dan de huidige op chemische stoffen gebaseerde evaluatie.

Voor de vergelijking van de meer klassieke LC_{50} (of EC_{50}) gebaseerde methoden met de meer recente TKTD analyse is daarom naar synthetisch chemische stoffen in de EFSA pesticiden database gekeken en zijn daaruit de stoffen met een beperkt risico geselecteerd. Dit resulteerde in 14 stoffen, waarvoor voldoende effectwaarden in de tijd in databases konden worden gevonden om TKTD modellen toe te passen en een drempelwaarde voor effecten af te leiden. Voor de meeste stoffen met een beperkt risico waren onvoldoende gegevens beschikbaar om een drempelwaarde voor effecten af te leiden. Voor twee stoffen konden voldoende drempelwaarden voor effecten op verschillende organismen worden afgeleid om een SSD (Species Sensitivity Curve) te genereren. In de risicobeoordeling wordt een SSD gebruikt om een concentratie af te leiden die effect heeft op 5% van de soorten en daarmee 95% van de soorten beschermt. Deze waarde is voor één van de stoffen veel lager dan het toelatingscriterium uit de risicobeoordeling voor deze stof, maar is een factor 7 hoger dan de waterkwaliteitsnorm voor deze stof uit de Kaderrichtlijn Water (KRW). Als deze laatste waterkwaliteitsnorm wordt gehanteerd, zijn alle organismen op de SSD curve beschermd.

De conclusie uit dit onderzoek is dat de "proof-of-concept" om TKTD modellen te gebruiken in de risicobeoordeling werkt: met TKTD modellen kunnen drempelwaarden voor effecten worden afgeleid, die leiden tot waarden die een grotere bescherming bieden dan de normen uit een standaard-risicobeoordeling. Echter, de toepassing van deze modellen in de risicobeoordeling voor laag-risico middelen is niet mogelijk voor microbiële middelen en feromonen en dient nader te worden onderzocht voor natuurlijke plantenstoffen die als gewasbeschermingsmiddel worden gebruikt.

Summary

ToxicoKinetic ToxicoDynamic (TKTD) approaches are now becoming more widely used. This development was stimulated by the EFSA scientific opinion on TKTD modelling published in 2015. A TKTD approach is different in its nature and scientific assumptions than standard approaches based on LC_{50}s or EC_{50}s. The most important difference is that such an assessment can be based on time-independent parameter values of which the Threshold for Effects is the most important parameter value. The effect threshold is a measure for the intrinsic toxicity of the exposed organism to the compound of interest and by definition this is the concentration below which no effects occur even after prolonged exposure time (or in other words the LC_0 at infinite exposure time).

TKTD models require experimental observations on effects at different points in time. These time specific data are the basis of a TKTD approach but are generally not available in the open literature. Also, in this research the lack of reported effect data over time reduced the possibilities to use TKTD based approaches. Effect thresholds are generally lower than the LC_{50}s on which current RA is based. Thus, the question arises if sufficient data are available to derive these effect thresholds and if an assessment based on effect thresholds is still protective.

The TKTD approach was applied to compounds used in crop protection that are classified as 'limited' risk for aquatic organisms. Most low-risk compounds are biological by nature and therefore not suitable for a TKTD approach. Compounds with limited risk (compounds with a H412 or H413 hazard classification) were selected from the EFSA pesticide database, which resulted in 14 synthetic chemical compounds. It showed that for most compounds insufficient data were available to derive effect thresholds. However, effect thresholds could be estimated for a number of species/compound combinations. Of the 14 compounds, Acetamiprid is the most interesting as this is an insecticide with enough data available to create an SSD based on effect thresholds. Acetamiprid shows a high variation in its toxicity and especially *Chironomus* appears to be very sensitive to acetamiprid. The HC_5 that was derived from the SSD was 0.72 µg/L. This value is substantially lower than the admission criterium (100 µg/L) used in the pesticide directive. It showed that the admission criterium does not protect 35% of the potentially exposed species. The Environmental Quality Standard (0.1 µg/L) derived in the context of the Water Framework Directive turned out to be protective; the EQS is a factor of 7 below the HC_5. With an SSD based on LC_{50}s the difference between the EQS and the HC_5 would be a factor of around 20.

So as proof of concept an assessment based on effect thresholds is feasible and will generally lead to lower values than standard risk assessment and might in the case of acetamiprid have led to a slightly lower EQS than the one that is currently used. Such an approach takes away most drawbacks from an LC_{50} or EC_{50} based approaches and lower assessment factors may be used.

1 Introduction

1.1 Risk Assessment of chemicals

Current Risk Assessment (RA) is based on (mostly) standardised tests carried out in the laboratory with the focus on lethal or sub-lethal endpoints. The most common sub-lethal endpoints are effects on growth and/or reproduction.

The summary statistic that is used is typically an LC_{50} (the concentration that kills 50% of the exposed organisms at some specified point in time) for lethal endpoints or an EC_{50} (the concentration with 50% effect on growth or reproduction, also at some specified point in time) for sublethal endpoints. In addition, No Observed Effect Concentrations (NOECs), specified for some point in time can be used. This is the highest concentration at which effects are not statistically different from the effect in the controls. The NOEC is often mistakenly interpreted as the concentration that shows no effect, but a statistical analysis showed that for a well-designed and expertly carried out experiment the NOEC equates to app 25% effect (Hoeven, 1998). The main problem with the NOEC approach is that absence of proof of effects is not the same as proof of absence and therefore NOECs are heavily criticized in the scientific literature for having fundamental problems, e.g. (Crane & Newman, 2000; Jager, 2012; Laskowski, 1995).

The 50% effect mark is derived from the dose/response curve, which is usually a sigmoidal curve (Ritz, 2010), see Figure 1 for an example, taken from (Aighewi & Ishaque, 2014).

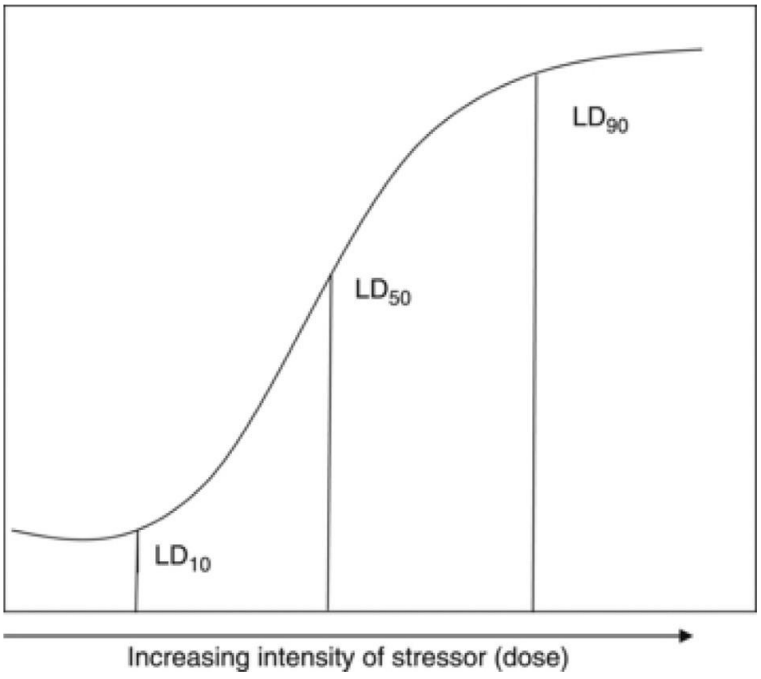

Figure 1 *Example of a Dose response curve. LD stands for lethal dose.*

For RA a 0% effect would be preferable, but with the approach with a sigmoidal dose/response curve this will lead to infinitely small exposure concentrations as the 0 percent effect point is not defined. The 50% effect point however is very well defined and different sigmoidal curves will all pinpoint the 50% effect concentration. The 10% effect concentration can in most cases also be derived from the dose/response curve and this concentration is increasingly used in RA.

As stated, the summary statistics of a test (LC_{50}, EC_{50}, EC_{10}) are only valid for exposure on specific point in time and are usually strongly time-dependent, or in other words depend on the duration of the exposure to a substance (Baas, Jager, & Kooijman, 2010; Heckmann, Baas, & Jager, 2010; Jager, Heugens, & Kooijman, 2006). Therefore, acute and chronic tests were developed, the acute tests (typically an acute test lasts a few days) usually focus on effect on mortality and the chronic tests (these typically last several weeks) are generally more focussed on sub-lethal effects e.g. (ECHA specific guidance document R.7B; EFSA, 2013).

RA is intended to avoid adverse effects in the environment; so, a safe concentration for the environment must be derived from the test results, therefore assessment factors (AFs) are used; basically, the lowest EC_{50} from tests with different taxonomic groups is divided by an AF to derive a concentration with an acceptable risk. Different types of legislation (Pesticide regulation, Water Framework Directive) can use different AFs. In the Water Framework Directive (WFD) these AFs depend on the amount of toxicity data available. In the pesticide directive (EC, 2009; 2013) AFs are provided in the data requirements (EC, 2013) or for higher tier data in the Aquatic Guidance document (EFSA, 2013).

1.2 Different Types of Regulations

Pesticide regulation
In Europe, the authorisation of pesticides on the market is regulated by Regulation (EC) No 1107/2009 (EC, 2009). The risk assessment to support this authorisation of pesticides is performed at two levels: EFSA (European Food Safety Authority) authorises active substances used in pesticide products, while Member States authorize formulated products at national level. EFSA (2013; Aquatic Guidance document) is intended to be used for the authorisation of both active substances and formulated plant protection products.

The pesticide directive follows a tiered approach, where lower tiers include standardized and general tests. These tests are generally more conservative, are less time-consuming and less expensive than higher tier studies, while the latter are more realistic, more specific but also often more labour-intensive and expensive. All tiers aim to assess the same protection goal. The pesticide directive clearly distinguishes the risks of short-term from those of long-term exposure. The directive has its focus on relatively small bodies of water and small areas of land and their inhabiting organisms in the direct vicinity of the agricultural fields – the so-called edge-of-field water bodies and off-field habitats- (Brock et al., 2013).

In the lower-tier risk assessment, acute toxicity data are divided by maximum predicted exposure concentration (PECmax) values to generate acute toxicity exposure ratios (TERshort-term). The TERshort-term should be at least 100 for the most sensitive animal species and at least 10 for the most sensitive primary producers, which are numbers that can be considered as assessment factors (AFs). Chronic NOEC values are divided by either the PECmax or the predicted time-weighted average concentration (PECTWA) values to derive chronic TERs (TERlong-term). Table 1 gives an overview of standard test species and their RAC (Regulatory Acceptable Concentration or admission criterium) derivation in the lower-tier risk assessment.

Table 1 *Standard test species in the lower-tier risk assessment for aquatic organisms (EFSA, 2013 GD; EFSA, 2015 Sediment opinion).*

	Standard test species	Duration	Insecticide	Herbicide	Fungicide	RAC
Acute	*Daphnia*	48 h	X	X	X	EC50/100
	Additional arthropod (*Chironomus* or *Americamysis bahia*)	48 h	X			EC50/100
	Fish (*Oncorhynchus mykiss*)	96 h	X	X	X	LC50/100
Chronic	Algae (green)	72 h	X	X	X	ErC50/10
	Algae (non-green e.g. diatom)	72 h		X		ErC50/10
	Macrophyte (*Lemna, Myriophyllum, Glyceria*)	7 - 14 d		X		ErC50/10
	Fish ELS or FLC test		X	X	X	EC10/10 or NOEC/10
	Daphnia or additional arthropod	21 d	X	X	X	EC10/10 or NOEC/10
	Chironomus, Hyalella, Lumbriculus	28 d	X	X	X	EC10/10 or NOEC/10

Higher tiers are informed by the lower tier risk assessment and should focus on the most sensitive organism(s) in the lower tier risk assessment. Tier 2 includes acute or chronic laboratory tests with additional species and/or refined exposure. Data from these additional species tests might be combined in a Species Sensitivity Distribution (SSD) or be combined with modelling. Tier 3 includes population and community level experiments and models, while Tier 4 focuses on field studies and landscape level models.

EFSA (2013; Aquatic guidance document) give guidance on the Assessment Factors (AFs) to be used in higher tier tests. For SSDs these can vary from 3 to 6. For mesocosm studies the AF differs between deriving an ETO-RAC (Ecological Threshold option) or an ERA-RAC (Ecological Recovery Option) from a mesocosm study. For an ETO-RAC an AF of 2 – 3 is required while for an ERO-RAC the AF varies from 3 – 5. The number of species data used to fit the distribution has to be adequate from a statistical point of view. A SSD that addresses the sensitivity of fish should be based on a minimum of 5 toxicity data points from the viewpoint of animal welfare, for other organisms this minimum is 8 (EFSA, 2013).

Water Framework regulation

Since 2000, the Water Framework Directive (WFD) (https://environment.ec.europa.eu/topics/water/surface-water_en) has been the main legal instrument for water protection in Europe. Together with the Environmental Quality Standards Directive and the Groundwater Directive. It applies to inland, transitional and coastal surface waters, as well as groundwaters. It ensures an integrated approach to water management, respecting the integrity of entire ecosystems, including by regulating individual and setting corresponding regulatory standards. According to the Directives, the Commission is under a legal obligation to regularly review these lists of pollutants. For most pesticides the Water Framework Directive (WFD) pollutants and setting corresponding regulatory standards. In the Environmental Quality Standards Directive (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02008L0105-20130913) a list of Environmental Quality Standards (EQS) is presented for a range of pollutants, including pesticides.

This leads to the situation that the pesticide regulation can have different quality standards for surface waters than those that apply in the WFD. These differences are mainly caused by differences in available information as the quality standards from the WFD come later in time and therefore allow to consider additional relevant literature.

Regulation for other chemicals than pesticides

Currently, apart from pesticides, every chemical that gets on the market must be assessed within the European legal framework Registration Evaluation Authorisation and Restriction of Chemicals (REACH). For pesticides this is not relevant, but there are similarities in the line of reasoning and therefore we mention the REACH regulation here.

The environmental assessment is generally based on chemical tests with daphnids, fish and or algae. When these three groups are tested there is ecotoxicological data available for some different biological groups,

covering at least some biodiversity. If data for several species belonging to several biological groups are available a species sensitivity distribution (SSD) can be made. Species Sensitivity Distribution Curves are also an option in the higher tiers of the Pesticide Directive and the Water Framework Directive. A SSD is a sigmoidal curve where the 5% cut-off concentration plus an adequate assessment factor is used as the environmentally safe concentration (HC_5) in all the three regulations mentioned above. The SSD approach has taken some criticism for being biased (Forbes & Calow, 2002), however it is considered to be a reliable approach to derive safe environmental concentrations and takes into account effects on larger numbers of species (there is still debate on the minimally required number of species, ECHA and WFD recommend some 10 different species (ECHA guidance document R.10)), while the pesticide directive requires at least 8 datapoints in a SSD except for fish where for animal welfare reasons a number of 5 datapoints is considered as sufficient. So, this requires extensive ecotoxicological testing, which is generally not carried out. Note that this approach specifically allows to affect 5% of the potentially exposed species.

Depending on the number of tests available, the REACH regulations prescribe different AFs, depending on which data is available, see Table 2 (ECHA guidance document R.10). Basically, an assessment factor is a number (5, 10, 100, 1000) through which the LC_{50} is divided to derive safe environmental concentration. This assessment factor is supposed to consider the extrapolation from 50% to 0 (or 5%) effect but also the intra- and inter-species variation.

Table 2 *Overview of assessment factors depending on available information as applied in REACH and the WFD.*

AF	Data available
1000	At least one short-term L(E)C50 from each of three trophic levels (fish, invertebrates (preferred Daphnia) and algae)
100	One long-term EC10 or NOEC (either fish or Daphnia)
50	Two long-term results (e.g., EC10 or NOECs) from species representing two trophic levels (fish and/or Daphnia and/or algae)
10	Long-term results (e.g., EC10 or NOECs) from at least three species (Normally fish, Daphnia and algae) representing three trophic levels
1-5	Species sensitivity distribution (SSD) method

The clear general idea is that the less ecotoxicological information is available the higher the assessment factor or in other words a lower concentration is allowed in the environment. In the pesticide risk assessment (EFSA, 2013), for SSDs with aquatic invertebrates and/or primary producers AFs of 3 – 6 are applied in the risk assessment (Table 27 in EFSA, 2013) while for vertebrates this is 3 – 9 (Table 28 in EFSA, 2013), while the AF also depends on the acute or chronic nature of the SSD and the endpoint taken from the SSD (either HC5 or Lower Limit).

1.3 Low risk pesticides

The EU Chemicals Strategy for Sustainability (https://environment.ec.europa.eu/strategy/chemicals-strategy_en) sets aims for use of chemicals as part of the EU's zero pollution ambition, which is a key commitment of the European Green Deal; The EU's chemicals strategy aims to: better protect citizens and the environment; boost innovation for safe and sustainable chemicals; require substances with a lower basic risk profile.

Within regulation 1107/2009 an active substance can be approved as a low-risk substances if it meets the regular approval criteria and the low-risk criteria in Annex II, point 5 of Regulation (EC) 1107/2009 (in accordance with Regulation (EC) No 1272/2008). There are specific criteria for chemical substances and for micro-organisms. Products that contain only low-risk substances can then be authorised as a low-risk plant protection product.

A basic substance is an active substance which (following regulation 1107/2009 article 23): is not a substance of concern; and does not have an inherent capacity to cause endocrine disrupting, neurotoxic or immunotoxic effects; and is not predominantly used for plant protection purposes but nevertheless is useful in plant protection either directly or in a product consisting of the substance and a simple diluent; and is not placed on the market as a plant protection product.

Low-risk substances and basic substances are substances expected to have a lower basic risk profile. Annex II, point 5 of Regulation (EC) 1107/2009 gives the following criteria for low-risk active substances:

An active substance shall not be considered of low risk where it is or must be classified in accordance with Regulation (EC) No 1272/2008 as at least one of the following:
- carcinogenic,
- mutagenic,
- toxic to reproduction,
- sensitising chemicals,
- very toxic or toxic,
- explosive,
- corrosive.

It shall also not be considered as of low risk if:
- persistent (half-life in soil is more than 60 days),
- bioconcentration factor is higher than 100,
- it is deemed to be an endocrine disrupter, or
- it has neurotoxic or immunotoxic effects.

1.4 How can TKTD models be helpful in the risk assessment?

A Toxico Kinetc Toxico Dynamic (TKTD) approach is very different from the standard approaches based on LC_x or EC_x values, in its nature and scientific assumptions.

The difference that is most eye catching is that this is a process-based approach where uptake and elimination are explicitly considered, and effects are also described by a specified mechanism (Baas, Augustine, Marques, & Dorne, 2018; Jager, Albert, Preuss, & Ashauer, 2011; Jager et al., 2006; Kooijman et al., 2009). The basic premise is that effects can only occur after a compound is taken up by the organism and once the compound is taken up effects may occur. Therefore, the approach consists of a specified uptake and elimination module; the toxico kinetic part (usually based on the one-compartment model) and a specified module to translate the exposure to effects; the toxico dynamic part. In principle, the assumptions and approaches are valid and applied to both aquatic species and terrestrial species.

Cadmium is a well-known example where traditional TIER 1 approaches fail to assess the high toxicity of Cadmium for a variety of different organisms. Cadmium often has a slow uptake and therefore concentrations build up slowly in an organism. In a traditional acute test with daphnids (2 days), fish (4 days) or honeybees (2 days) for example, one would conclude that Cadmium is hardly toxic as high exposure concentrations are needed to induce an effect. This however is not caused by an intrinsically low toxicity of Cadmium but because of slow kinetics e.g. (Heard et al., 2017). Basically, the slow uptake of Cadmium appears to indicate a low toxicity because effects are still building up. If the standard acute test would be extended to longer periods of time it will show that Cadmium is indeed highly toxic for many species, but this just does not show in a short-term test and not necessarily in a chronic test.

Here the focus is on lethal effects for aquatic (or terrestrial species). TKTD models for the interpretation of effects on aquatic macrophytes or plants or sub-lethal effects do not have an approved status (yet) and will not be considered here.

For the interpretation of survival data (lethal effects) the General Unified Threshold model of Survival (GUTS) was developed (Jager et al., 2011) and acknowledged as a model for the interpretation of data on survival by

EFSA (EFSA et al., 2018). The GUTS modelling framework can be used in different ways, depending on the amount of available data. The full version of GUTS uses observed internal concentrations as a driver for effects. This can only be used if detailed internal and external concentrations over time are available. This is rarely the case. Therefore, the reduced GUTS model is generally the basis for the interpretation of the data. The reduced GUTS model is based on environmental concentrations as the driving force for effects. The model uses three parameters to calculate the survival probability of an individual, given an exposure scenario. The parameter values are derived from an ecotoxicological test, just like the LC_{50} is calculated.

An LC_{50} is calculated by feeding the exposure concentrations together with observed effects at some specified point in time in a log-logistic model and the resulting LC_{50}, including a 95% Confidence Interval (CI) is calculated by the model. With the GUTS approach the exposure concentrations together with the observed for all points in time are fed into the model and the resulting parameter values, including the 95% CI are calculated by the model. The model parameters are:

- The Effect Threshold or No Effect Concentration
- The dominant rate constant
- An additional TD parameter, describing effects

The Effect Threshold is the most important parameter value as this is a measure for the intrinsic toxicity of the exposed organism to the compound of interest (Baas & Kooijman, 2015); this is by definition the concentration below which no effects occur even after prolonged exposure or in other words the LC_0 at infinite exposure time. The other parameters are important for understanding the effects over time and to make extrapolations to other exposure situations as effects can be calculated for any exposure concentration (pulsed, declining over time, etc, etc) (Roman Ashauer et al., 2016; R. Ashauer & Escher, 2010). By definition an LC_{50} is defined for a constant exposure concentration which in real life hardly ever happens. This reduces the possibility to extrapolate the laboratory results to real life exposures dramatically. For example, it is impossible to say if a 96-hr exposure to a concentration of 5 µg/L will have any effect if the 48 hr LC_{50} equals 10 µg/L. Or the other way around; it is impossible to say if a 24-hr exposure to a concentration of 20 µg/L will have any effect if the 48 hr LC_{50} equals 10 µg/L. Or if a 48-hr exposure to a concentration starting with 20 µg/L declining with some first order rate constant will have any effect if the 48 hr LC_{50} equals 10 µg/L. Even comparing the sensitivity of species based on LC_{50} values should be carried out with care as the time dependence of the LC_{50} may be different for different species (Heard et al., 2017; Jager et al., 2006).

A TKTD approach has no problem with questions like these, any exposure profile can be used to derive parameter values and if parameter values are known predictions on effects can be made for any other exposure profile. In addition, if LC_{50} s (or for example LC_{50} s) are known for at least 3 points in time the GUTS parameters can be calculated, and predictions can be made. The effect threshold (or LC_0) is an excellent starting point for further RA. This parameter is time-independent and the extrapolation from an LC_{50} to an LC_0 with (i.e., which is supposed to be factored in, in the AFs) is no longer needed (Jager et al., 2006).

So overall the TKTD approach has superior extrapolation potential and starts with an effect threshold as a measure for the sensitivity of a species, which is derived from the model. The downside is that observations on effects are needed at different points in time and these time specific data are not always available. The effect thresholds (LC_0) will naturally be lower than the LC_{50}s, which raises the question on whether the current legislation would still be protective when an assessment would be based on effect thresholds from TKTD models instead of LC_{50}s.

1.5 Aim of the research

This research was intended to evaluate the 'Risk' for compounds that are classified as low risk or 'limited' risk and are used in crop protection by applying a TKTD approach to assess the effects on aquatic organisms. The results of this exercise are evaluated and compared to the results obtained by traditional approaches of RA, with the final aim to show the feasibility of an approach based on the use of effect thresholds derived with TKTD model application.

The following steps were taken in this research:

1. Check existing lists of approved low risk active ingredients or products (NL, EU): Which chemical substances are on that list? Are there any ecotoxicological effect test data available?
2. If (raw) ecotoxicological effect test data are available for fish or invertebrates, GUTS models can be used to derive the effect threshold for that chemical/species combination (based on the open GUTS software).
3. Where possible Species Sensitivity Distributions were calculated.

Note the general approaches and regulations are applied both for aquatic and terrestrial RA. However, in a first TIER RA the assessment is based on aquatic organisms. The focus of this research is also on aquatic RA, mostly because here most data are available. This does not imply that the application of TKTD approaches is limited to aquatic organisms.

2 Choice of compounds

Starting point was the EFSA Pesticides Database - Active Substances (File created on 2021-10-14). This database contains all active substances listed for use in Europe.

This database lists a total amount of 1461 compounds of which 454 are approved and 33 are listed as low risk.

2.1 Low risk compounds

The low-risk compounds are generally compounds that have a natural occurrence; examples are bacteria strains, viruses, plant hormones, yeast-based compounds or extracts from tree bark. In addition, some chemical compounds like CaCO3, $NaHCO_3$, Ureum and some iron salts like $FeCl_3$ and $FePO_4$.

Most of these compounds are widely used and several of these compounds have a use in food products. As such they do not pose an ecotoxicological threat, but they can have an effect on some species. The iron salts for instance are used as a compound to prevent snails from causing damage to crops or keep them out of places where they are unwanted. Part of these compounds fall under the basic substances and part are microbial pesticides under the pesticide directive. The pesticide directive has a separate part for microbial pesticides that has recently been adopted (21 November 2022), see: https://food.ec.europa.eu/plants/pesticides/micro-organisms and the uniform principles and the data requirements have been adapted for microbial pesticides, i.e. pesticides that contain micro-organisms as its active ingredient instead of chemical molecules.

None of the compounds that were categorised as low risk were suitable for an interpretation with the classical LC_{50} based approaches or TKTD approaches. Therefore, chemical pesticides were selected which are categorized as having limited aquatic risk (instead of having low risk).

2.2 Limited risk compounds

Pesticides have a hazard classification for aquatic risk, ranging from category 1 (very poisonous for aquatic organisms) to category (could have long term harmful effects for aquatic organisms), see Table 3. So, this is basically a ranking of compounds on their intrinsic toxicity for aquatic organisms. Compounds that have a H412 or H413 hazard classification were selected for further evaluation. In addition, some compounds do not have a classification at all, which also implies that such a compound does not pose a hazard for aquatic organisms and therefore these compounds were also selected.

Table 3 *Overview of hazard classifications for the aquatic environment (https://echa.europa.eu/nl/information-on-chemicals/cl-inventory-database/-/discli/details/60842).*

H400	Acute danger for the aquatic environment, danger category 1	"Very poisonous for aquatic organisms."
H410	Chronic danger for the aquatic environment, danger category 1	"Very poisonous for aquatic organisms, with long-term effects."
H411	Chronic danger for the aquatic environment, danger category 2	"Poisonous for aquatic organisms, with long-term effects."
H412	Chronic danger for the aquatic environment, danger category 3	"Harmful for aquatic organisms, with long-term effects."
H413	Chronic danger for the aquatic environment, danger category 4	"Could have long-term harmful effects for aquatic organisms."

Based on this starting point a stepwise selection of relevant compounds was made:

1. Start with all EFSA registered and market allowed active compounds (1461 compounds)
2. Select all active compounds that are 'approved' (454 compounds)

3-1. Select active compounds with classification H412 of H413 (11 compounds)

3-2. Select active compounds without classification for environmental effects (14 compounds)

2.2.1 Compounds with H412/413 classification

In Table 4 an overview of compounds with a H412 or H413 classification are listed. In addition, the available water quality criteria (environmental standard and the admission criterium are listed and it is indicated if the water quality criteria are exceeded in the Netherlands in 2020, based on the results summarised in the 'bestrijdingsmiddelenatlas'. This is a website where measured concentrations in surface waters for pesticides are listed in the Netherlands and where it is indicated whether the current Environmental Quality Standards are exceeded (https://www.bestrijdingsmiddelenatlas.nl/atlas/1/1).

Table 4 *Overview of compounds with a H412 or H413 classification with their environmental and admission criteria and observed exceedances of quality criteria.*

Stofnaam	H class.	EQS (µg/L)	Exceedance of env standard in 2020	Admission Criterium (µg/L)	Exceedance of admission criterium in 2020
2,4-D	H412	26	No	34.6	No
Acetamiprid	H412	0.1	Yes	100	No
Bentazone	H412	73	No	54	No
Clethodim	H412	1	No	12.1	No
Dicamba	H412	0.13	No	45	No
Diflufenican	H412	9	No	0.085	No
Fluroxypyr	H412	1100	No	27.6	No
Hymexazol	H412	8.8	No	40	No
Isoxaben	H413	0.11	Yes	3.75	No
Metalaxyl	H412	9.7	No	100	No

2.2.2 Compounds without classification

Non-classified compounds are mainly low risk compounds but there are a few additional pesticides, see Table 5. This is surprising as a classification would be expected for pesticides.

Table 5 *Overview of compounds without classification with their environmental and admission criteria and observed exceedances of quality criteria.*

Compound name	EQS (µg/L)	Exceedance of env standard in 2020	Admission criterium (µg/L)	Exceedance of admission criterium in 2020
Acetic acid	na	na	na	na
Benzoic acid	na	na	na	na
Calcium carbide	na	na	na	na
Carvone	na	na	na	na
Ethylene	na	na	na	na
Hydrogenperoxide	na	na	na	na
Ironsulfate	na	na	na	na
Sulphur	na	na	na	na
Flonicamid	120	No	310	No
Chlormequat	500	No	240	No
Metalaxyl-M*	9.7	No	100	No
Dichlorprop-P	1	No	-	-
Clopyralid	75	No	990	No
Cycloxidim	2.6	No	-	-

3 TKTD approach for the chosen compounds

3.1 Materials and methods TKTD approach

The TKTD modelling approach, based on the GUTS modelling framework was used to check if any further analysis of the available data was possible and if this then would lead to different conclusions with regards to environmental standards. In order to do this ecotoxicological data are needed. The ECOTOX database hosted by the US-EPA (https://cfpub.epa.gov/ecotox/) was used as a starting point in a stepwise approach:

1. List of LC_x of LD_x values for all aquatic organisms;
2. The lowest reported value is selected to obtain the result for the most sensitive species;
3. Wherever possible relevant LC_{50} values are used to derive Effect thresholds with a TKTD approach;
4. For the insecticides an additional literature search was conducted with the aim to extract additional data from literature that is not present in the US-EPA database in order to derive additional effect thresholds as basis for an SSD.

As a final step, the Effect thresholds and the lowest reported ecotoxicological value were compared with the admission criterium and the EQSs that are in place for the aquatic environment.

3.2 Results for compounds classified as H412 or H413

3.2.1 Data found in the EUS-EPA ECOTOX database

The results of the evaluation of compounds classified as H412 or H413 are summarised in Table 6. The table lists the lowest effect value that is listed in the Ecotox database and the lowest Effect threshold that could be derived with the TKTD model.

Note that the TKTD Effect threshold could only be derived if at least three LC_{50}s at different points in time are listed. This combination of data was available for a limited nr of compounds and species and therefore does not cover all aquatic species and for some compounds an effect threshold could not be derived at all.

Table 6 *Result of the evaluation.*

Compound name	ECOTOX Entries	Lowest value (µg/L)	Type	Species for which the lowest value was found	TKTD Lowest Effect threshold (µg/L) and species for which it could be derived
2,4-D	298	14 (juvenile) 86900 (adult)	48 h LC_{50}	*Oreochromis niloticus*	2976.9 *Rasbora neilgherriensis*
Acetamiprid	118	0.52	LC_{10}	*Chironomus dilutus*	2.2 *Hyalella azteca*
Bentazone	17	34400	48 h LC_{50}	*Chironomus riparius*	
Clethodim	13	19000	96 h LC_{50}	*Oncorhynchus mykiss*	30765.3 *Palaemonetes africanus*
Dicamba	51	1000	48 h LC_{50}	*Leiostomus xanthurus*	2100 *Gammarus fasciatus*
Diflufenican					
Fluroxypyr	5	14300	48 h LC_{50}	*Bluegill*	-
Hymexazol	13	40	48 h LC_{50}	*Several species*	-
Isoxaben	5	870	96 h LC_{50}	*Cyprinodon variegatus*	
Metalaxyl (mixture)	23	730	96 h LC_{50}	*Americamysis bahia*	

3.2.2 Additional literature search for the insecticides

Search terms: acetamiprid; LC_{50} (in title); LC_{50} in keywords or text and aquatic in keywords or text.

		Ecotox;NEC
Gambusia Holbrooki	https://doi.org/10.1111/wej.12549	4 LC50s; 31 mg/L
Procambarus clarkii	ETC, 36, pp. 2838–2848, 2017	4 LC50s; 1.7 mg/L
Clarias gariepinus	https://doi.org/10.1080/03601234.2020.1763712	96 h LC5 232 ppm
Hyalella azteca	https://doi.org/10.1016/j.ecoenv.2019.03.038	28 d EC10 3.3 ug/L
Hexagenia spp	https://doi.org/10.1016/j.envpol.2018.03.004	96 h EC25 55 ug/L
Biomphalaria alexandrina	Env Sci and Pol Res (2018) 25:32582–32590	24 h LC0 2.3 mg/L
Hyalella azteca	This research, see Table 6	2.2 ug/L

3.2.3 SSD generation

For 2,4-D sufficient data was available to derive an SSD based on Monte Carlo simulations on the basis of effect thresholds based on *Arthropoda* and fish and an HC5 could be estimated from these data, which is shown in Figure 2.

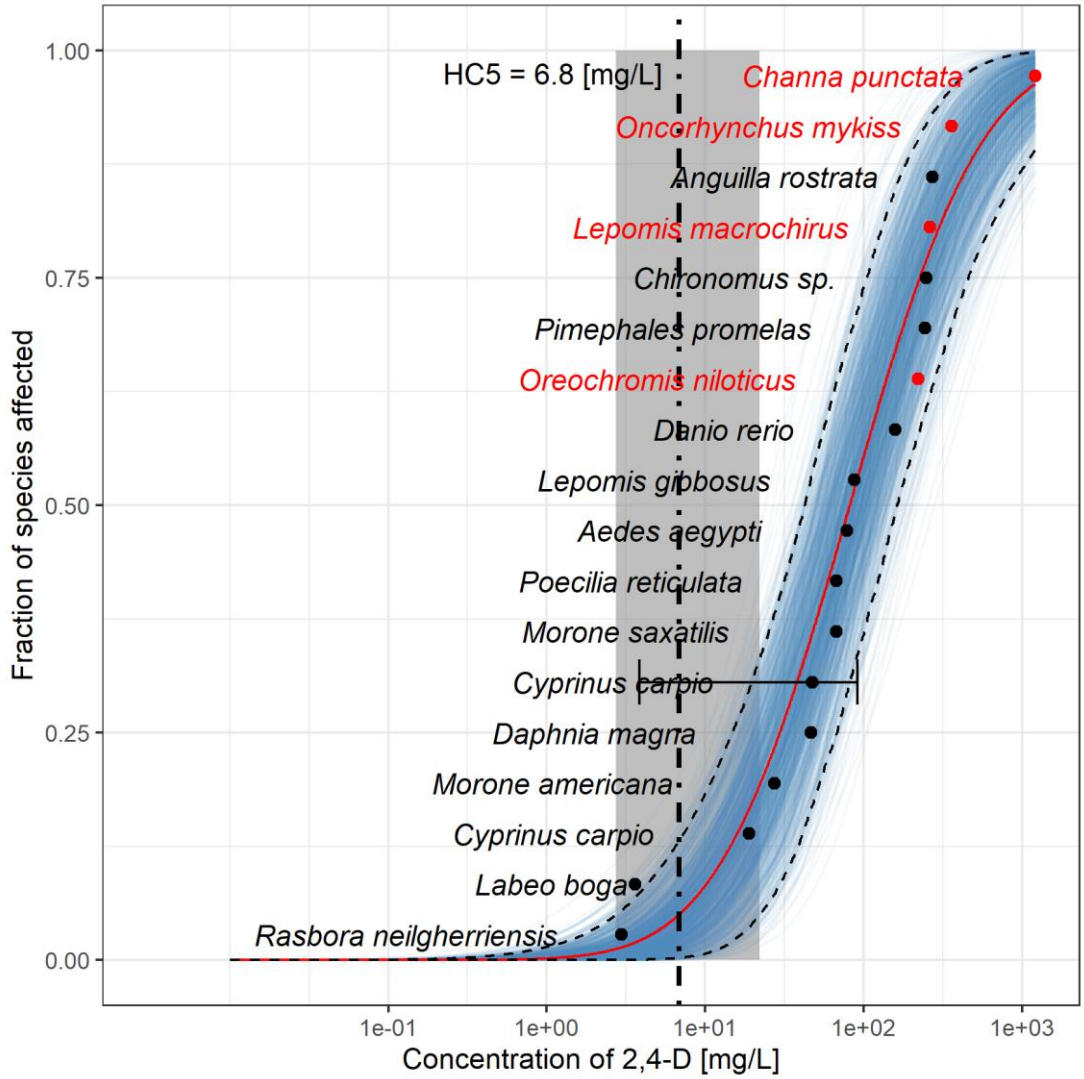

Figure 2 *SSD derived with a TKTD approach for Arthropoda and fish for 2,4-D. The red line gives the median value of 1000 Monte Carlo simulations (blue lines) following the TKTD approach. The dotted line gives the 95%-CI around the median value. Different species are indicated with a black dot if an effect threshold could be estimated and with a red dot if the value was estimated based on available data. The vertical dotted line gives the median HC5 value of 6.8 mg/L, the grey area gives the 95%-CI around the median value for HC_5 (note that the horizontal axis is on a log scale).*

For Acetamiprid an SSD based on effect thresholds could also be made, this is shown in Figure 3. The calculated HC_5 value is 0.72 µg/L.

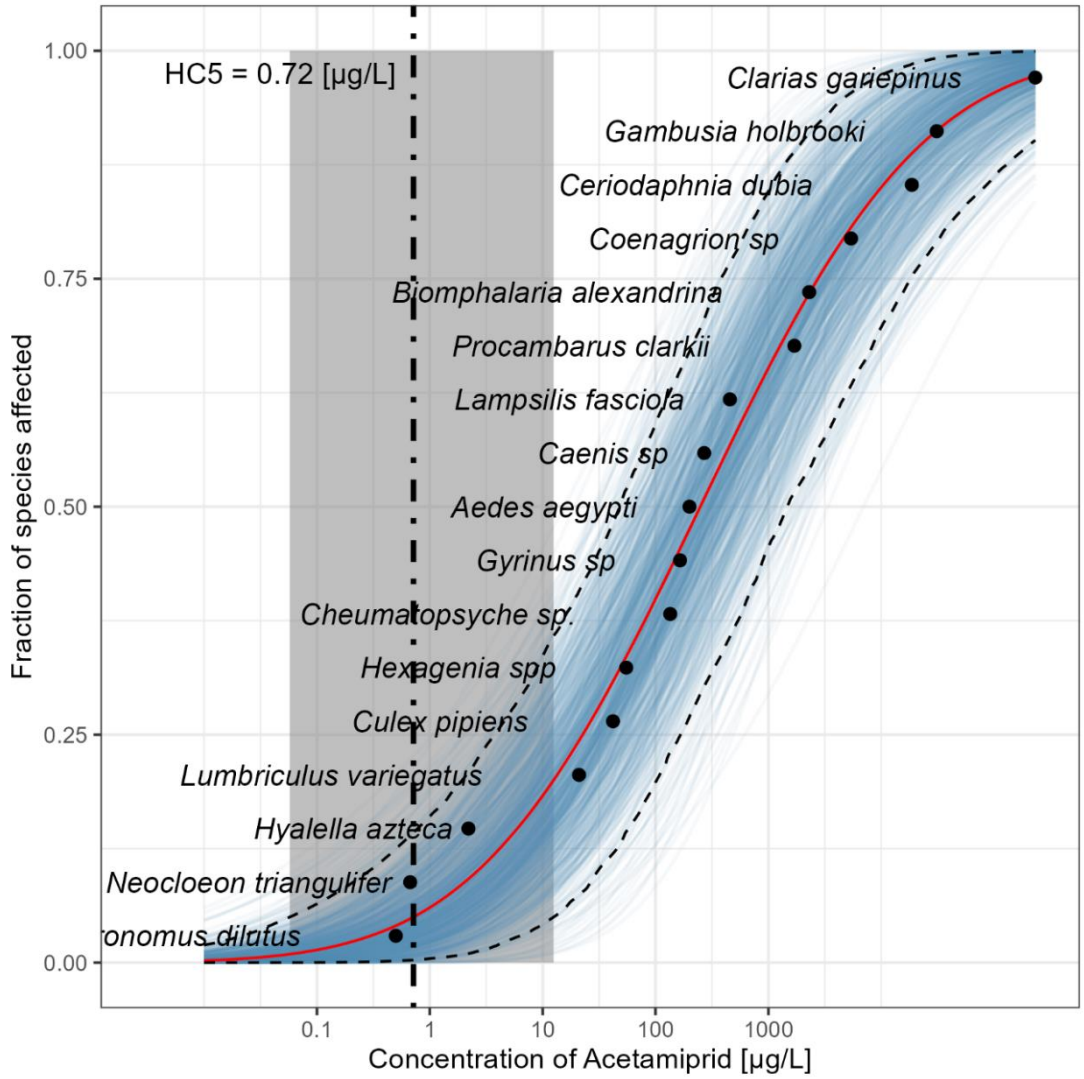

Figure 3 *SSD for Arthropoda and fish for acetamiprid. The red line gives the median value of 1000 Monte Carlo simulations (blue lines). The dotted line gives the 95%-CI around the median value. Different species are indicated with a black dot if an effect threshold could be estimated and with a red dot if the value was estimated based on available data. The vertical dotted line gives the median HC_5 value of 0.72 µg/L, the grey area gives the 95%-CI around the median value for HC_5 (note that the horizontal axis is on a log scale).*

3.3 Results for compounds without classification

3.3.1 Data found in the EUS-EPA ECOTOX database

Results of the evaluation for compounds without classification are summarised in Table 7.

Table 7 *Result of the evaluation.*

Stofnaam	Ecotox entries	Lowest value (µg/L)	type	Soort	TKTD
Acetic acid	na	na	na	Na	na
Benzoic acid	na	na	na	Na	na
Calcium carbide	na	na	na	Na	na
Carvone	na	na	na	Na	na
Ethylene	na	na	na	Na	na
Hydrogenperoxide	na	na	na	Na	na
Ironsulfate	na	na	na	Na	na
Sulphur	na	na	na	Na	na
Flonicamid	4	97900	4 d LC50	*Oncorhynchus mykiss*	-
Chlormequat	15	80000	4 d LC50	*Nitocra spinipes*	-
Metalaxyl-M*	6	41900	2 d LC50	*Daphnia magna*	-
Dichlorprop-P	9	500	4 d LC50	*Oncorhynchus mykiss*	-
Clopyralid	8	448000	4 d LC5	*Oncorhynchus mykiss*	-
Cycloxidim*	0	-	-	-	-

* Cycloxidim also not available in the PPDB.

Note: Chlormequat is classified in the US as a 'low risk' growth regulator. However, Chlormequat is also classified as an 'extremely hazardous substance' resulting in strict regulations on production, use and storage.

3.3.2 Additional literature search

For Flonicamid an additional literature search was conducted: flonicamid (keyword); LC_{50} in keywords or text. This led to only one additional hit (see below) so a further evaluation was not possible.

		NEC
Cyprinus carpio	https://doi.org/10.22363/2312-797X-2019-14-3-279-288	42.5 mg/L

4 Discussion

4.1 TKTD approach

For the compounds that are classified as 'low risk' a TKTD approach was not feasible or just not practical. For the compounds classified as H412, H413 or for those which did not receive a classification, a TKTD approach proved to be principally feasible. However due to lack of effect data over time, an effect threshold could only be calculated for a limited number of species/compound combinations. In none of the cases effect data at different points in time were available for the species with the lowest LC_{50}. Where effect thresholds could be calculated, it showed that these were up to a factor of 4 lower than the LC_{50} values.

For Acetamiprid and 2,4-D sufficient data were available to generate an SSD based on effect thresholds. Since 2,4D is a herbicide and the GUTS based TKTD models are intended to be used for animals and not for plants, the insecticide Acetamiprid is the more interesting example. Acetamiprid shows a high variation in its toxicity and especially *Chironomus* appears to be very sensitive to acetamiprid. It showed that the HC_5 value that was derived from the SSD for acetamiprid is 0.7 µg/L, so the survival of *Chironomus* (LC10 = 0.52 µg/L) is not protected by the HC_5 value that was derived from the SSD.

As proof of concept an assessment based on effect thresholds is feasible and will generally lead to lower values than standard risk assessment. Such an approach takes away most drawbacks from an LC_{50} (time dependence, 50% effect) or EC_{50} based approach, which was discussed in the introduction.

4.2 Comparison of the results with Environmental standards

4.2.1 Comparison with the Pesticide Directive

For none of the compounds the admission criterium was exceeded in Dutch surface waters in 2020. The admission criterium was generally significantly higher than the lowest LC_{50} found in the US-EPA Ecotox database, except for hymexazol and acetamiprid. For Hymexazol the admission criterium (40 µg/L) is exactly the same as the lowest reported LC_{50} for several species.

But most striking is the comparison for acetamiprid. For this compound the admission criterium equals 100 µg/L and the lowest reported LC_{10} equals 0.52 µg/L. The HC_5 based on the Effect thresholds equals 0.72 µg/L. A comparison with the SSD shows that the admission criterium does not protect some 35% of the potentially exposed species (see Figure 3).

So here an assessment based on classical approaches would also lead to the conclusion that the admission criterium is not protective for a number of species. With the TKTD approach this becomes more pronounced.

4.2.2 Comparison with the Water Framework Directive

For acetamiprid and isoxaben the EQS was exceeded in Dutch surface waters in 2020. Most EQS values are over a factor of 10 below the lowest LC_{50}, so sufficiently protective.

There are 3 compounds, 2,4-D, acetamiprid and hymexazol, where the lowest LC_{50} is a factor of five higher than the EQS (implying an assessment factor of 5). Most eye catching is the very low reported LC_{50} value for the juvenile *Oreochromis niloticus* exposed to 2,4-D. Here the reported LC_{50} is even below the EQS. This LC_{50}, originally published by Tejada et al. in 1994 (Tejada et al., 1993) is considered to be an outlier. The difference between the sensitivity of the adults and the juveniles is striking and not plausible. Indeed, all other entries for juvenile *Oreochromis niloticus* exposed to 2,4-D are significantly higher, ranging from

60 – 222 mg/L for a 2 d LC_{50} value. So, this value is discarded and the EQS for 2,4-D is sufficiently protective.

For acetamiprid and hymexazole the difference between the lowest effect concentration and the EQS is below a factor of 5. This was the reason to re-evaluate the EQS, especially since we are evaluating effects on survival and sub-lethal effects generally occur in lower concentration ranges than lethal effects. For hymexazole relatively little effect data is available and for acetamiprid enough species were available to create an SSD based on effect thresholds.

When the HC_5 for acetamiprid is compared to the EQS, it shows that the EQS is a factor of 7 below the HC_5. This would give an assessment factor of 7 to generate protectivity but also here sub-lethal effects may occur in lower concentration ranges. An SSD based on LC_{50} values results in an assessment factor around 20, which would be sufficiently protective.

4.2.3 Differences between the WFD and the Pesticide directive

Generally, for the compounds that were taken up in this analysis the Admission Criterium used in the pesticide directive is in the same order of magnitude as the EQS used in the WFD. The EQS generally takes into account more data than those submitted for the pesticide directive and therefore is generally lower (more protective). However, there are a few distinct differences, most striking are the differences between the EQS and the admission criterium for acetamiprid (a factor 1000) and dicamba (a factor of 350). On the other hand, the admission criterium is much stricter for diflufenican (a factor of 105) and fluroxypyr (a factor of 40).

Acetamiprid is the most interesting compound as here very large differences in the sensitivity of different species are observed. If the selection of species to be tested is narrow it is easy to miss a very sensitive species, leading to a high value for the admission criterium.

It is not clear why the admission criterium is much stricter than the EQS for diflufenican and fluroxypyr. Diflufenican does not have any entry in the US-EPA Ecotox Database. Effect concentrations reported in the Pesticide Property Database show effect concentrations between 12 and 50 µg/L (for fish, aquatic invertebrates and sediment dwelling organisms), but an EC_{50} for algal growth of 0.25 µg/L; all values are higher than the admission criterium of 0.085 µg/L (but lethal effects occur in concentrations well below the EQS of 9 µg/L). Fluroxypyr has 5 entries in the US-EPA Ecotox database, the lowest effect value (14300 µg/L) is still well above the admission criterium of 27.6 µg/L.

5 Conclusions

For the evaluation and comparison of TKTD approaches against the more classical chemical approaches the focus was put on chemical active ingredients that are categorized as compounds with a limited risk.

TKTD approaches are now becoming more widely used and for effects on survival of fish and invertebrates a modelling framework was made available and accepted for regulatory use by EFSA. This is the GUTS model, and this was used in this assessment. A TKTD approach for the interpretation of toxic effects is very different from the standard approaches based on LC_{50}s or EC_{50} values. The most important difference is that an assessment can be based on time-independent parameter values of which the Effect Threshold (by definition, the LC_0 for infinite exposure time) is the most important parameter value as this is a measure for the intrinsic toxicity of the exposed organism to the compound of interest. With the effect threshold as a starting point for risk assessment, intrinsically RA is based on 0% effects and not on 50% effects which is a different starting point allowing to use lower assessment factors than those currently used.

The effect thresholds are generally lower than the LC_{50} values, which raises the question if sufficient data are available to derive these effect thresholds and if an assessment based on effect thresholds is still protective within the current legislative framework. The approach was applied to compounds that are used in crop protection that are classified as low risk or 'limited' risk for aquatic organisms.

The exercise showed that for most compounds insufficient data were available in the open literature to derive effect thresholds. However, effect thresholds could be estimated for several species/compound combinations. Acetamiprid is the most interesting compound as this is an insecticide with enough data available to create an SSD based on effect thresholds. Acetamiprid shows a high variation in its toxicity between organisms and especially *Chironomus* appears to be very sensitive to acetamiprid. The HC_5 that was derived from the SSD was 0.72 µg/L.

This value is substantially lower than the admission criterium (100 µg/L) used in the pesticide directive. It showed that the admission criterium does not protect about 20% of the potentially exposed species. When the HC_5 for acetamiprid is compared to the EQS (0.1 µg/L), it shows that the EQS is a factor of 7 below the HC_5. An SSD based on LC_{50} values would give an assessment factor around 20, which would be sufficiently protective.

As proof of concept an assessment based on effect thresholds is feasible and will generally lead to lower values than a standard risk assessment and in the case of acetamiprid might have led to a slightly lower EQS than the one that is currently used. Such an approach will take away most drawbacks from an LC_{50} or EC_{50} based approach.

However, the majority of low-risk compounds are biological in their origin. These can be natural compounds like pheromones but also bacteria, viruses or fungi that can be used as an active ingredient against pests. By their biological origin these compounds are not suitable for a Risk Assessment based on chemicals, such as the commonly used LC_{50} or EC_{50} approaches or the more recently accepted TKTD approaches. For example, if a pest species becomes infected with a virus, the virus multiplies in the host organism and as such affects the host organism. Regardless of the approach, chemical evaluations assume some equilibrium between the environmental concentration and the concentration inside the organism. So the basic starting assumption for a RA based on chemicals fails for biology based active ingredients. This implies that new tools need to be developed for low risk compounds with a biological nature.

6 Outlook

In this report we have shown that there are no principal limitations for the application of TKTD models in the Risk Assessment of classical chemical pesticides.

The advantages of a TKTD approach over standard approaches using e.g., an LC_{50} or EC_{50} are:

- Time independent parameter values, providing a better proxy for the sensitivity of species than the currently used LC_x values.
- The toxicity parameters can be derived from any exposure profile, not necessarily a constant exposure over time which is a pre-requisite for the derivation of an LC_{50} from laboratory data.
- If parameter values are known, effect predictions can be made for any (time-dependent) exposure profile that may occur under field conditions.

The downside is that effect observations are needed at different points in time, which is more labour intensive then a standard toxicity test. Note that for e.g., daphnids and honeybees, its already a requirement to observe survival at intermediate time points, but only the 48 hr LC_{50} is mentioned as the result of the test in the available literature.

Based on the example of Acetamiprid, a discussion meeting with Ctgb could be organized for addressing the question if from regulatory side such additional safety assessment, which would be possible without additional data requirements and with a relative low effort, would be considered as reasonable and an useful addition to the requirements for (low-risk or limited-risk) substances.

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The mission of Wageningen University & Research is "To explore the potential of nature to improve the quality of life". Under the banner Wageningen University & Research, Wageningen University and the specialised research institutes of the Wageningen Research Foundation have joined forces in contributing to finding solutions to important questions in the domain of healthy food and living environment. With its roughly 30 branches, 7,600 employees (6,700 fte) and 13,100 students and over 150,000 participants to WUR's Life Long Learning, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.