oekotoxzentrum
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Schweizerisches Zentrum für angewandte Ökotoxikologie
Centre Suisse d'écotoxicologie appliquée
Eawag-EPFL

Praxistaugliche Beurteilungen von kurzzeitigen Expositionsspitzen

Endbericht

25.09.2017

Impressum

Herausgeber

Oekotoxzentrum, Schweizerisches Zentrum für angewandte Ökotoxikologie Eawag-EPFL,
8600 Dübendorf

Im Auftrag von

Bundesamt für Umwelt (BAFU) 3003 Bern
Das BAFU ist ein Amt des Eidg. Departements für Umwelt, Verkehr, Energie und Kommunikation (UVEK).

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Danksagung

Für die Bereitstellung der Monitoringdaten aus der NAWA SPEZ 2015-Studie möchten wir dem ganzen NAWA SPEZ-TEAM aber insbesondere den teilnehmenden kantonalen Fachstellen und der Abteilung Umweltchemie der Eawag danken. Darüber hinaus möchten wir folgenden Wissenschaftlern und Wissenschaftlerinnen für schnelle und unkomplizierte Auskunft zu ihren Studien danken: Pierre-Jean Copin und Nathalie Chévre von der Universität Lausanne, Wiebke Drost vom deutschen Umweltbundesamt und Annika Agatz, von der Universität York.

Hinweis

Dieser Bericht wurde im Auftrag des Bundesamtes für Umwelt (BAFU) verfasst. Für den Inhalt sind allein die Auftragnehmer verantwortlich. Dieser Bericht ist eine Interpretation und Zusammenfassung des englischen Berichts “Effect modelling to evaluate water quality monitoring strategies” [1]

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Zitiervorschlag

Junghans M., Kuhl R., Zimmer E., Ashauer R., Werner I. (2017) Praxistaugliche Beurteilungen von kurzzeitigen Peaks. Studie im Auftrag des BAFU. Oekotoxzentrum Eawag-EPFL, Dübendorf. 2014.

Titelfoto: Andri Bryner, Eawag

Zusammenfassung

Im Beurteilungskonzept für Mikroverunreinigungen aus diffusen Quellen wurde vorgeschlagen, Qualitätskriterien zum Schutz vor chronischen Effekten (CQK) mittels zeitproportionaler 14-Tage Mischproben zu überprüfen. Im Hinblick auf die geplante Verankerung von CQK als numerische Anforderungen in der Schweizerischen Gewässerschutzverordnung (GSchV), sollte überprüft werden, ob dieser Zeitraum geeignet ist, um chronische Risiken für Wasserorganismen zu beurteilen. In dieser Studie wurde anhand von Modellsimulationen und realen Expositionsdaten untersucht, ob Mischproben die im Feld zu erwartende Toxizität gut abbilden oder eher zu Unter- bzw. Überschätzungen der Toxizität führen. Die Ergebnisse der Modellierungen bestätigen die Eignung des Zeitintegrals von 14 Tagen für die Gewässerüberwachung. Obwohl es über den gesamte Zeitraum des Expositionsprofils auch zu Unter- und Überschätzungen der vorhergesagten Toxizität kommt, stimmt die Vorhersage für die toxischsten Zeiträume sehr gut mit der für die Mischprobe modellierten Toxizität überein. Die Abweichungen sind für jede untersuchte Organismengruppe im Mittel geringer als Faktor 2. Es muss aber bedacht werden, dass die Modellierungen auf nur 7 Arten, 7 Wirkstoffen und 5 Probenahmestellen beruhen. Ausserdem wurde neben der Mortalität für Bachflohkrebse und Fische nur eine kleine Auswahl an sub-letalen Effekten betrachtet, nämlich Reproduktion und Längenwachstum bei Wasserflöhen sowie Populationswachstum bei Wasserlinsen und Algen. Die Ergebnisse zeigen auch, dass Qualitätskriterien zum Schutz vor akuten Effekten (AQK) mittels zeitproportionaler 3-Tage Mischproben überprüft werden können.

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1 Einleitung

Dieser Bericht ist eine Interpretation und Zusammenfassung des englischen Berichts “Effect modelling to evaluate water quality monitoring strategies” [1].

1.1 Hintergrund

Mit der folgenden Änderung der Gewässerschutzverordnung (GSchV, SR 814.201) vom 01.01.2016 wurde die Möglichkeit eröffnet, in Anhang 2 GSchV ökotoxikologisch basierte numerische Anforderungen an die Wasserqualität für oberirdische Gewässer festzulegen (Anh. 2 Ziff. 11 Abs. 1 Bst. f GSchV):

„*Die Wasserqualität muss so beschaffen sein, dass […] Stoffe, die durch menschliche Tätigkeit ins Gewässer gelangen, die Fortpflanzung, Entwicklung und Gesundheit empfindlicher Pflanzen, Tiere und Mikroorganismen nicht beeinträchtigen“.*

Zu diesem Zweck wurden für gewässerrelevante Stoffe je zwei numerische Anforderungen hergeleitet: eine, mit der überprüft werden kann, ob akute Risiken für die Gesundheit von Gewässerorganismen bestehen (akutes Qualitätskriterium, AQK) sowie eine zur Feststellung chronischer Risiken für Gewässerorganismen (chronisches Qualitätskriterium, CQK), z.B. in Bezug auf eine Beeinträchtigung der Fortpflanzung oder der Entwicklung.

Bislang gelten alle numerischen Anforderungen in Anhang 2 der GSchV zu jeder Zeit. Die Einführung eines AQK und eines CQK macht es aber erforderlich, dass mit ihnen auch eine Zeitspanne definiert wird, in denen sie nicht überschritten sein dürfen.

Unter der EU-Wasserrahmenrichtlinie sollte das akute Qualitätskriterium nie überschritten werden. Das chronische Qualitätskriterium sollte im Jahresmittel nicht überschritten werden oder, für Stoffe mit stark fluktuierenden Konzentrationen wie Pflanzenschutzmittel, im Mittel des Zeitraums ihres Auftretens im Gewässer. Die Überprüfung von Letzterem ist aus verschiedenen Gründen schwer umzusetzen. Zum einen müsste bei jeder Monitoringkampagne und für jedes Gewässer ein eigener Zeitraum für jeden Stoff festgelegt werden, was nicht nur aufwendig ist, sondern auch die zeitliche und räumliche Vergleichbarkeit der Ergebnisse erschwert. Zum anderen ist unklar, ob damit der von der GSchV geforderte Schutz der Gewässerorganismen hinreichend gewährleistet wäre.

Aus diesen Gründen wurde im Beurteilungskonzept für organische Spurenstoffe aus diffusen Einträgen ein effektbasierter Ansatz zur Festlegung der Zeitspannen vorgeschlagen [2]. In einer ersten Näherung wurde angenommen, dass der Effekt auf aquatische Organismen davon abhängt, wie lange diese einer bestimmten Konzentration ausgesetzt sind (Habers Gesetz zitiert in [3]). Das Prinzip ist in Abbildung 1 dargestellt. Es wird davon ausgegangen, dass die Effektstärke durch das Produkt aus Zeit und Konzentration bestimmt wird. Daher sollte eine zeitgewichtete Durchschnittskonzentration unabhängig vom Expositionsmuster denselben ökotoxikologischen Effekt bewirken.

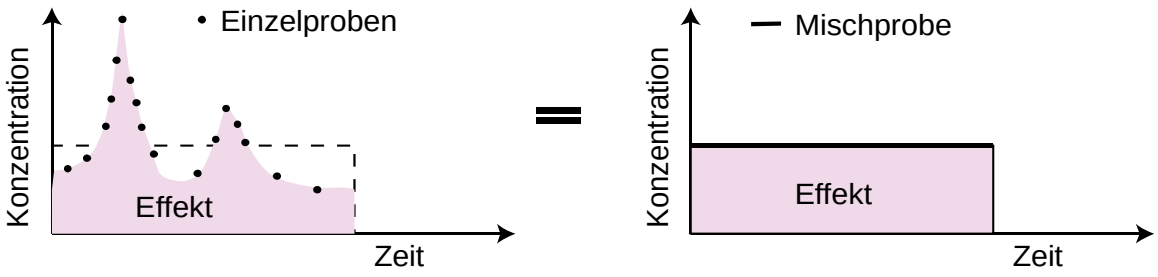

***Abb. 1:** Schematische Abbildung des Zusammenhangs: „ökotoxikologischer Effekt = Zeit x Konzentration“. Dies bedeutet, dass das Zeitintegral (rechts) den gleichen chronischen Effekt repräsentiert wie viele Einzelproben (links).*

Die Frage ist demnach, welche Zeitdauer einer chronischen Exposition entspricht. Bei der Pflanzenschutzmittelzulassung wird als Standard eine Zeitdauer von einer Woche vorgeschlagen [3]. Allerdings wird darauf verwiesen, dass sich die Bestimmung des Zeitraumes idealerweise an der Dauer des chronischen Tests orientieren soll, der für die Beurteilung herangezogen wird.

Für die Herleitung von chronischen Qualitätskriterien werden Testdaten verwendet, bei denen die Organismen bestimmten Substanzen zwischen 72 Stunden und mehreren Wochen einer konstanten Konzentration ausgesetzt werden (Abbildung 2). Die Zeitdauer der Tests, die zur Herleitung der CQK herangezogen werden, entspricht also in etwa einem Zeitintegral von 2 Wochen.

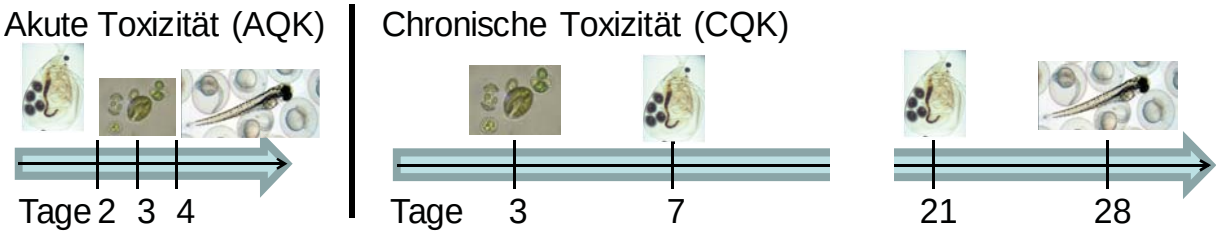

Abb. 2: *Übersicht über die Zeitdauer der verschiedenen Tests welche zur Herleitung von akuten (links) und chronischen (rechts) Qualitätskriterien verwendet werden. Für Fische werden 28 Tage als minimale Zeitdauer für einen chronischen Test definiert. Je nach Fischart, Endpunkt und Testsubstanz können auch längere Tests zur Verfügung stehen.*

Daher wurde von Wittmer et al. (2014) vorgeschlagen, die CQK mittels kontinuierlicher zeitproportionaler 14-Tage-Mischproben zu überprüfen. Das analoge Zeitintervall für den AQK entspricht 3 Tagen. Allerdings ist in der aktuellen Änderung der GSchV vorgesehen, dass die AQK zu keiner Zeit überschritten werden dürfen (siehe dazu auch Diskussion in der Schlussfolgerung).

Mit der vorliegenden Studie soll anhand von computerbasierter Effekt-Modellierung überprüft werden, ob dieses Vorgehen auf reale Monitoringdaten anwendbar ist. Es sollten hauptsächlich zwei Fragen geklärt werden: (1) mit welchen Zeitspannen sollten AQK und CQK verglichen werden und (2) wie gross sind die Abweichungen zwischen der vorhergesagten Toxizität durch die zeitlich gemittelten Konzentrationen und jener die durch das reale Expositionsprofil zu erwarten ist. Die Analyse basiert auf toxikokinetischen-toxikodynamischen Modellen [4, 5] für den Bachflohkrebs (*Gammarus pulex*), den Wasserfloh (*Daphnia magna*) und die Dickkopfelritze (*Pimephales promelas*) sowie auf Populationsmodellen für Wasserlinsen und Algen. Die Modelle wurden anhand von Toxizitätsdaten kalibriert. Alle Modelle berücksichtigen Erholung in Phasen niedrigerer Expositionskonzentrationen.

2 Material und Methoden

2.1 Modellierungen

Die Modellierungen werden in den Anhängen A bis C des englischen Berichtes [1] beschrieben.

Als Expositionsprofile für die Modellierung wurden Daten aus der NAWA-SPEZ Monitoringkampagne aus dem Jahr 2015 verwendet [6, 7]. Diese Daten wurden aus zwei Gründen ausgewählt: Zum einen wurden dort kleine Bäche in landwirtschaftlich intensiv genutzten Gebieten beprobt, die starke Fluktuationen der Belastung mit Pflanzenschutzmittelwirkstoffen zeigen. Zum anderen wurde dort über mehrere Monate kontinuierlich beprobt und die Probenahmezeiträume daraufhin optimiert, dass auch Konzentrationsspitzen erfasst werden können. Die maximale zeitliche Auflösung betrug 12h. Während Trockenperioden wurden aber auch Proben über mehrere Tage und Wochen genommen.

Für jedes Expositionsprofil wurden Zeitfenster von 14 und 3 Tagen simuliert.

Von den über 100 gemessenen Stoffen wurden die Profile von sieben Pflanzenschutzmittelwirkstoffen für die Modellierungen ausgewählt (Tabelle 1).

Tab. 1: *Für die Modellierungen ausgewählte Stoffe mit ihrem Wirkmechanismus. Zusätzlich sind jeweils noch die empfindlichen Organismengruppen angegeben. Diese Angabe beruht auf den validen und relevanten Ökotoxizitätsdaten aus den Stoffdatenblättern zu den Qualitätskriterienvorschlägen des Oekotoxzentrums*[1]*.*

Stoff	**Chemische Klasse**	**Wirkort (Wirkmechanismus)** [8]	**Empfindlichste Organismengruppe(n)**
Fungizide			
Carbendazim	Benzimidazole	Zellteilung (Hemmung der beta-Tubulin-Synthese)	Krebstiere und Fische
Herbizide			
Diuron	Phenylharnstoffe	Energiegewinnung (Hemmung der Photosynthese am Photosystem II)	Blaualgen und Algen
Metazachlor	Chloracetanilide	Zellwandbildung (Hemmung der Bildung sehr langkettiger Fettsäuren)	Grünalgen und höhere Pflanzen
Insektizide			
Chlorpyrifos	Organophosphate	Nervensystem (Hemmung der Cholinesterase)	Krebstiere und Insekten
Diazinon	Organophosphate	Nervensystem (Hemmung der Cholinesterase)	Krebstiere
Dimethoate	Organophosphate	Nervensystem (Hemmung der Cholinesterase)	Insekten und Krebstiere
Imidachloprid	Neonicotinoide	Nervensystem (Bindung an postsynaptische Nicotinoid-Rezeptoren im zentralen Nervensystem von Insekten)	Insekten und Krebstiere

1 http://www.oekotoxzentrum.ch/expertenservice/qualitaetskriterien/qualitaetskriterienvorschlaege-oekotoxzentrum/ Die empfindlichste Organismengruppe steht jeweils an erster Stelle.

Tabelle 2 fasst zusammen, für welche Kombinationen aus Organismus, Expositionsprofil und Substanz Toxizitätsprofile modelliert wurden. Es wurden alle Stoff- und Ortkombinationen modelliert für die Expositionsprofile vorlagen. Wenn ein Stoff für einen Ort nicht modelliert wurde (weisses Feld), wurde der Stoff an dem Ort nicht gemessen.

Tab. 2: *Überblick über die modellierten Organismus-, Expositionsprofil- und Substanzkombinationen. Modellierte Kombinationen sind grau unterlegt. Die Zahlen geben den Endpunkt an, für den die Toxizität jeweils modelliert wurde: (1) Mortalität von Bachflohkrebsen (Gammarus pulex), (2) Mortalität von Fischen (Pimephales promelas), (3) Reproduktion von Wasserflöhen (Daphnia magna), (4) Wasserlinsenwachstum (Lemna gibba bzw Lemna minor) und (5) Algenwachstum (unterschiedliche Arten).*

Stoff	**Canale Piano di Magadino**		**Eschelisbach**		**La Tsatonire**		**Mooskanal**		**Weierbach**	
Mortalität von Bachflohkrebs (1) & Fisch (2)										
Carbendazim			**1**		**1**				**1**	
Chlorpyrifos			**1**	**2**	**1**	**2**				
Diazinon			**1**	**2**					**1**	**2**
Dimethoate					**1**				**1**	
Mortalität von Bachflohkrebs (1) & Fisch (2), Reproduktion von Wasserflöhen (3)										
Imidachloprid			**1**		**3**	**1**		**3**			**1**		**3**
Wachstum von Wasserlinsen (4) & Algen (5)										
Diuron			**4**	**5**	**4**	**5**			**4**	**5**
Metazachlor	**4**	**5**					**4**	**5**	**4**	**5**

2.2 Effektdaten

2.2.1 Mortalität von Bachflohkrebsen und Fischen

Für das Fungizid Carbendazim und alle vier Insektizide (Chlorpyrifos, Diazinon, Dimethoate, Imidacloprid) wurde die Mortalität des Bachflohkrebs *Gammarus pulex* modelliert. Die Effektdaten stammen aus bereits veröffentlichten Studien [8-10] oder noch unveröffentlichten Studien (Carbendazim und Dimethoat). Für Chlorpyrifos und Diazinon waren genügend Daten für die Dickkopfelritze (*Pimephales promelas*), einem Süsswasserfisch, vorhanden [11, 12], und so konnte zusätzlich noch die Mortalität dieser Fischart modelliert werden. Die Toxizitätsdaten stammen ebenfalls aus bereits veröffentlichten Studien. Alle Modellierungen basieren auf dem Modell „General Unified Threshold model of Survival“ (GUTS) [13].

2.2.2 Reproduktion und Entwicklung von Wasserflöhen

Für Imidacloprid konnten zusätzlich zum Endpunkt Mortalität auch noch die Endpunkte Reproduktion (Anzahl Nachkommen) und Längenwachstum der Nachkommen von Wasserflöhen (*Daphnia magna)* modelliert werden. Das Modell basiert auf dem Konzept dynamischer Energiebudgets [14] und wurde basierend auf veröffentlichten Toxizitätsdaten kalibriert [15].

2.2.3 Wachstum von Wasserlinsen und Algen

Für die Modellierungen des Wachstums von Wasserlinsen und Algen wurden zwei Herbizide ausgewählt, die jeweils unterschiedliche Wirkmechanismen haben: der Phenylharnstoff Diuron und das Chloracetanilid Metazachlor. Es wurde hier ein einfaches Populationsmodell verwendet, das auf exponentiellem Wachstums beruht. Die zugrundeliegenden Daten stammen aus Pflanzenschutzmittel-Zulassungsberichten [16, 17] sowie aus der öffentlichen Literatur [18, 19].

2.3 Skalierung auf wirksame Konzentrationen: „Margin of Safety“

Bei der Simulation der Toxizität von Expositionsprofilen sind die resultierenden Effekte oft 0%, wenn die Konzentrationen unter dem toxischen Bereich liegen, oder 100%, wenn die Konzentrationen über dem toxischen Bereich liegen. Dies liegt daran, dass echte Konzentrationen in Gewässern über mehrere Größenordnungen hinweg variieren. Damit man die Unterschiede zwischen den Simulationen besser untersuchen kann, wurden die Expositionsprofile hoch- bzw. herunterskaliert. Dies wurde basierend auf dem „Margin of Safety“ (MoS) Konzept gemacht [20]. Wenn der MoS grösser als 1 ist, ist kein Effekt zu erwarten, ist der MoS kleiner als 1 sind für das analytisch bestimmte Expositionsprofil toxische Effekte zu erwarten. In dieser Studie wurde jeweils der MoS basierend auf dem LC50 bzw. EC50 berechnet. Für die Modellierungen mit den Bachflohkrebsen und Fischen gibt der MoS also den Faktor an um den das Expositionsprofil für das jeweilige Zeitfenster (3 Tage bzw. 14 Tage) multipliziert werden muss, damit am Ende des Zeitfensters eine 50%ige Mortalität vorhergesagt wird. Für die Modellierungen des Lemna- bzw. Algenwachstums gibt der MoS analog den Faktor an, um den das Expositionsprofil für das jeweilige Zeitfenster erhöht oder verringert werden muss um am Ende des Zeitfensters eine 50%ige Reduktion der Biomasse im Vergleich zur unbehandelten Kontrolle vorherzusagen. Die MoS werden für jedes Zeitfenster getrennt für die unveränderten Expositionsprofile („fluktuierende Konzentrationen“) und für den zeitlich gewichteten Mittelwert („Time Weighted Average“, TWA) berechnet. Die Modellrechnungen mit TWA entsprechen einer zeitproportionalen Mischprobe. Für jedes Zeitfenster wird simuliert, dass zu Beginn des Zeitfensters unbelastete Organismen eingesetzt werden. Wenn man nun die beiden MoS vergleicht, kann man bestimmen, welche Art der Exposition am Ende des jeweiligen Zeitfensters die höhere Toxizität hervorruft.

3 Ergebnisse und Diskussion

3.1 Führt die Mischprobe zu Unter- oder Überschätzung der Toxizität?

Wenn man sich die toxischsten Zeitfenster anschaut, also jene mit den niedrigsten MoS Werten, sieht man, dass für Mischproben und fluktuierende Konzentrationen (unveränderte Expositionsprofile) in der Regel eine sehr ähnliche Toxizität vorhergesagt wird (Abbildung 3).

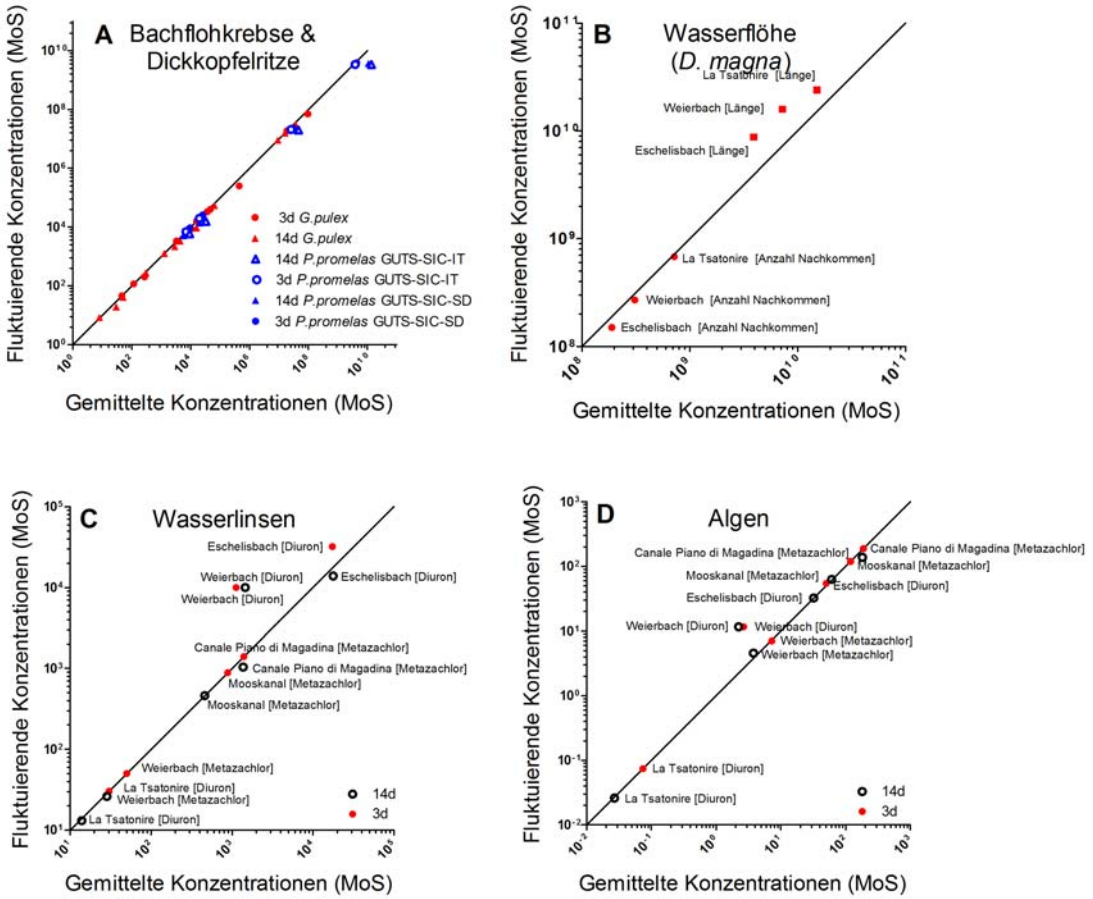

Abb. 3: *Vergleich der "Margin of Safety" (MoS) Werte basierend auf den zeitgewichteten Konzentrationsmitteln (TWA; stellvertretend für die Mischproben) mit jenen für die fluktuierenden Konzentrationen (unveränderte Expositionsprofile). Liegen die Datenpunkte oberhalb der diagonalen Linie handelt es sich um eine Überschätzung der Toxizität durch den zeitproportionalen Ansatz. Im umgekehrten Fall um eine Unterschätzung.*

Für Bachflohkrebse und Fische führt die Mischprobe eher zu einer Unterschätzung der Toxizität, vor allem für das 14-Tage Zeitfenster (Abbildung 5). Die Unterschätzung ist aber gering (im Mittel um einen Faktor 1.7 für das 14-Tage Zeitfenster). Einzig für einen Standort und nur für Fische wurde die Toxizität um mehr als einen Faktor 2 unterschätzt (Tabellen 3&4). Es handelt sich dabei um das toxischste 14 Tage Zeitfenster für Diazinon im Weierbach (Tabelle 4). Der Faktor von 2 wurde in Anlehnung an das Beurteilungskonzept gewählt. Erst eine Überschreitung des Qualitätskriteriums um mehr als einen Faktor 2 führt zu einer Einstufung des Gewässerzustands als unbefriedigend [2].

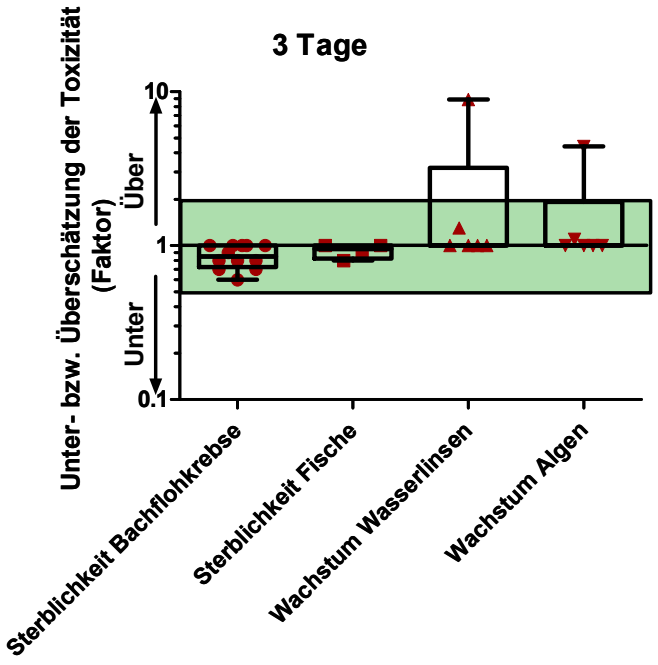

Abb. 4: *Über- und Unterschätzungen der fluktuierenden Konzentrationen durch die 3 Tages-Mischprobe. Grün = Über- und Unterschätzungen der Toxizität liegen innerhalb eines Faktors 2. Whiskers repräsentieren das 95ste Perzentil. Für die Fischsterblichkeit wurde der Mittelwert der Abweichungen aus den beiden TKTD-Modellen angegeben.*

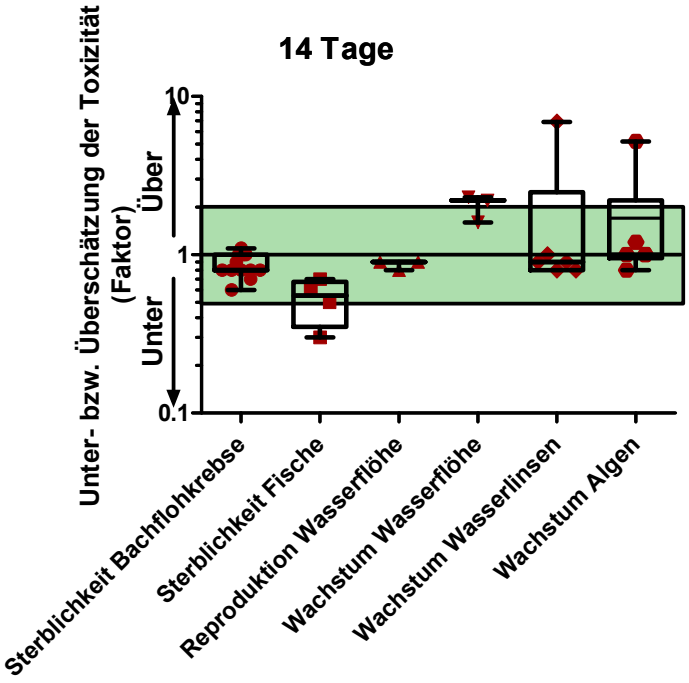

Abb. 5: *Über- und Unterschätzungen der fluktuierenden Konzentrationen durch die 14 Tages-Mischprobe. Grün = Über- und Unterschätzungen der Toxizität liegen innerhalb eines Faktors 2. Whiskers repräsentieren das 95ste Perzentil. Für die Fischsterblichkeit wurde der Mittelwert der Abweichungen aus den beiden TKTD-Modellen angegeben.*

Tab. 3: *Über- und Unterschätzungen der Toxizität durch die höchsten beobachteten Expositionskonzentrationen bei Verwendung eines 3 Tage-Mittelwerts. Grün = innerhalb eines Faktors 2, rot ↑ die Toxizität wird überschätzt, rot ↓ die Toxizität wird unterschätzt.*

Stoff	Canale Piano di Magadino	Eschelisbach	La Tsatonire	Mooskanal	Weierbach
Mortalität von Bachflohkrebs & Fisch					
Carbendazim					
Chlorpyrifos					
Diazinon					
Dimethoate					
Mortalität von Bachflohkrebs & Fisch, Reproduktion von Wasserflöhen					
Imidachloprid					
Wachstum von Wasserlinsen & Algen					
Diuron					↑ 8.9 / ↑ 4.4
Metazachlor					

Tab. 4: *Über- und Unterschätzungen der Toxizität durch die höchsten beobachteten Expositionskonzentrationen bei Verwendung eines 14 Tage-Mittelwerts. Grün = innerhalb eines Faktors 2, rot ↑ die Toxizität wird überschätzt, rot ↓ die Toxizität wird unterschätzt.*

Stoff	Canale Piano di Magadino	Eschelisbach	La Tsatonire	Mooskanal	Weierbach
Mortalität von Bachflohkrebs & Fisch					
Carbendazim					
Chlorpyrifos					
Diazinon					↓ 3.6
Dimethoate					
Mortalität von Bachflohkrebs & Fisch, Reproduktion von Wasserflöhen					
Imidachloprid					
Wachstum von Wasserlinsen & Algen					
Diuron					↑ 6.9 / ↑ 5.2
Metazachlor					

Für Wasserflöhe (*Daphnia magna*) werden nur die 14-Tage Zeitfenster gezeigt, da diese aufgrund des chronischen Endpunktes relevanter sind. Die Mischprobe für den Endpunkt Reproduktion (Anzahl Nachkommen) führt eher zu einer Unterschätzung der Toxizität und für die Körperlänge der Muttertiere eher zu einer Überschätzung (Abbildungen 3&5). Da der Endpunkt Wachstum (Körperlänge des Elterntiers) aber deutlich weniger empfindlich ist (höherer MoS), wird für die weitere Auswertung nur noch der Endpunkt Reproduktion berücksichtigt. In Abbildung 5 und Tabelle 4 sieht man, dass die Unterschätzung durch die Mischprobe gering ist (durchschnittlich um den Faktor 1.1).

Für Lemna und Algen sagen Mischprobe und fluktuierende Konzentration für das toxischste Zeitfenster in der Regel ebenfalls eine sehr ähnliche Toxizität vorher. Einzig für den Weierbach und Diuron gab es deutlich Überschätzungen der Toxizität bei Wasserlinsen und Algen, sowohl für das 3-Tage Zeitfenster, als auch für das 14-Tage Zeitfenster (Tabelle 3&4). Maximal überschätzte die Mischprobe die Toxizität für den Weierbach um den Faktor 8.9. Im Durchschnitt ist die Überschätzung der Toxizität durch die Mischproben aber vernachlässigbar (Faktor 1.25 für Wasserlinsen und Faktor 1.1 für Algen).

Vermutlich lässt sich diese Überschätzung durch das spezielle Expositionsprofil von Diuron im Weierbach (ein einzelner halbtägiger Peak) und den Endpunkt Wachstum erklären. Bei der Wachstumsmodellierung wurde die Annahme gemacht, dass die Toxikokinetik und die Toxikodynamik sehr schnell ist, so dass kurze Peaks auch nur zu kurzen Effekten führen. Dass diese Annahme für Diuron berechtigt ist, zeigt die Studie von Weber et al. [21] zur Populationsmodellierung für Algen in Gegenwart fluktuierender Konzentrationen von Isoproturon, einem Stoff, der strukturell mit Diuron verwandt ist und denselben Wirkmechanismus besitzt. Daher kann ein halbtägiger Peak das Populationswachstum auch nur kurze Zeit hemmen. Die Population wächst während der übrigen Tage wieder exponentiell, wenn Diuron wieder aus dem Organismus verschwindet. Es handelt sich dabei also um einen Erholungseffekt. Vermutlich gilt ein ähnlicher Zusammenhang für die Körperlänge des Elterntieres bei der Reproduktion von Wasserflöhen: das Wachstum wird während des Peaks ausgesetzt, kann dann aber wieder aufgenommen werden. In einer 3 Tage dauernden Mischprobe kann das Populationswachstum aber für längere Zeit gehemmt sein, selbst bei niedrigerer (Durchschnitts-)Konzentration, und so zu einer Überschätzung der Effekte führen. Beim Endpunkt Mortalität kann ein solcher Erholungseffekt nach kurzen Expositionspeaks nicht auftreten, da die während des Peaks gestorbenen Tiere weiterhin tot bleiben. Beim Endpunkt Reproduktion von Wasserflöhen gibt es vermutlich auch keinen Erholungseffekt, da die während des Peaks nicht produzierte Brut wohl auch am Ende des Zeitfensters immer noch fehlt.

3.2 Wie häufig kommt es zu Unter- oder Überschätzungen der Toxizität durch Mischproben?

Ein Vergleich über alle Zeitfenster zeigt, dass die Mischprobe für Bachflohkrebse und Fische die Toxizität häufiger unter- als überschätzt. Das gleiche gilt für den Endpunkt Reproduktion bei den Wasserflöhen. Für Wasserlinsen muss man zwischen den 3-Tage- und den 14-Tage-Zeitfenstern unterscheiden. In den vergleichsweise wenigen Fällen, wo sich die Toxizität am Ende des 3-Tage-Zeitfensters unterscheidet, kommt es ausschliesslich zu einer Überschätzung der Toxizität durch die Mischprobe. Für die 14-Tage Zeitfenster gibt es häufiger Abweichungen und die Mischprobe unterschätzt auch hier die Toxizität häufiger (28% der Zeit gegenüber 17% der Zeit). Für Algen halten sich die Über- und Unterschätzungen für das 3-Tage-Zeitfenster eher die Waage (12% der Zeit gegenüber 11% der Zeit). Für das 14-Tage-Zeitfenster kommt es eher zu Über- (36 % der Zeit) als zu Unterschätzungen der Toxizität durch die Mischprobe. Es ist aber zu beachten, dass es bei den Algen generell starke Unterschiede zwischen den Expositionsprofilen gibt.

Eine ausführlichere Auswertung inklusive Abbildungen ist im englischen Bericht enthalten [1].

4 Schlussfolgerungen

Generell zeigen die Modellsimulationen, dass zeitproportionale Mischproben zur Beurteilung der Toxizität fluktuierender Konzentrationen von Schadstoffen geeignet sind. Die im Beurteilungskonzept für Mikroverunreinigungen aus diffusen Quellen [2] vorgeschlagenen zeitproportionalen Mischproben über 14 Tage zur Überprüfung der CQK werden durch die Ergebnisse der Modellierungen als pragmatischer Ansatz bestätigt. Für Stoffe, deren chronische Toxizität ebenfalls durch den Endpunkt Mortalität bestimmt wird, sprechen die Ergebnisse eher noch für einen kürzeren Zeitraum. Auch für Stoffe, die vor allem die Reproduktion beeinträchtigen, könnte der Integrationszeitraum von 14 Tagen noch zu lang sein. Allerdings kann dazu noch keine abschliessende Einschätzung gegeben werden, da hier mit Imidacloprid nur ein Stoff in nur einer Art modelliert wurde. Für Stoffe, die vor allem das individuelle Wachstum (nicht aber das Populationswachstum) hemmen, kann das Zeitintervall von 14 Tagen in Einzelfällen die Toxizität überschätzen, nämlich dann, wenn Erholungszeiträume zur Verfügung stehen.

Die Ergebnisse zeigen auch, dass zeitproportionale Mischproben über 3 Tage geeignet sind, um akute Risiken kontinuierlich zu erfassen. Dabei muss aber bedacht werden, dass die höchste zeitliche Auflösung der für die Modellierung verwendeten Expositionsprofile nur 12h betrug. Wie die Studie von Leu et al. [22] zeigt, können Konzentrationsspitzen aber noch deutlich kürzer und damit in der Maximalkonzentration auch deutlich höher sein als eine zeitproportionale Halbtages-Mischprobe. Gerade für Stoffe, deren Wirkung hauptsächlich auf Mortalität beruht, kann daher nicht ausgeschlossen werden, dass die Toxizität durch eine 3-Tagesmischprobe stärker unterschätzt wird als die Ergebnisse dieser Studie erahnen lassen. Daher erscheint es sinnvoll, in der Gewässerschutzverordnung festzulegen, dass AQK zu keinem Zeitpunkt überschritten werden dürfen. Dies gilt vor allem auch für Probenahmestrategien, die auf Stichproben beruhen. In den letzten Jahren hat sich aber gezeigt, dass bei Stichproben die Möglichkeit besteht, dass wichtige Konzentrationsspitzen nicht erfasst werden. Daher werden vermehrt zeitproportionale Mischproben genommen [7, 23, 24]. Je höher man diese zeitlich auflöst, desto höher ist der Analyseaufwand. Für die Planung der Messkampagne SPEZ 2017 in 5 kleinen Gewässern wurde eine Auflösung von 3.5 Tagen über den gesamten Zeitraum von März bis September als durchführbar erachtet. Die Ergebnisse der vorliegenden Studie zeigen, dass sich diese Probenahmestratregie auch für die Beurteilung akuter Risiken eignet.

Es ist wichtig zu bedenken, dass die Ergebnisse dieser Studie den derzeitigen Stand des Wissens wiedergeben. Es wurden zwar die üblichen Endpunkte (Mortalität, Wachstum und Reproduktion) modelliert, aber nur eine kleine Auswahl an sub-letalen Effekten. Andere Effekte, wie die auf das endokrine System, epigenetische Effekte oder Verhaltensänderungen könnten für die chronische Risikobewertung von Mikroverunreinigungen sowie die Mischtoxizität und Wechselwirkungen mit anderen, nicht-chemischen Stressoren ökologisch ebenfalls relevant sein. Diese Aspekte konnten hier jedoch nicht berücksichtigt werden. Es wurden Arten aus allen drei Organismengruppen (Wirbeltiere, Wirbellose und Pflanzen) modelliert, aber es wurden insgesamt nur 7 Arten untersucht. Ausserdem wurden zwar 18 Expositionsprofile untersucht, aber nur von 5 Standorten. Es ist also nicht auszuschliessen, dass mit weiteren sub-letalen Endpunkten und/oder weiteren Arten sich das Gesamtbild noch etwas verschiebt. Zurzeit kann das aber nicht untersucht werden, da die Datengrundlage dazu fehlt.

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Study report

Effect modelling to evaluate water quality monitoring strategies

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Date: 24 August 2017

Sponsor: Swiss Centre for Applied Ecotoxicology (Oekotoxzentrum), Eawag-EPFL, Überlandstrasse 133, CH - 8600 Dübendorf, Switzerland

Project: This study refers to the contract “Praxistaugliche Beurteilung von kurzzeitigen Peaks” between Eawag and Bafu (00.0296.PZ / P292-0468).

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Introduction

This study aims to provide evidence for the Swiss authorities' development of new strategies for water quality monitoring. One question relating to water quality monitoring concerns the typically fluctuating nature of micropollutants, especially plant protection products, in water bodies and the question of what is the best water sampling strategy. Continuous time-proportional sampling of water bodies followed by chemical analysis of the pooled sample for the whole sampling duration has been proposed. Assuming that the chemicals do not degrade in the sampler this method will yield the time-weighted average (TWA) concentration over the sampling duration. If sampling times are equally spaced this corresponds simply to the average concentration.

Two questions arise in this context: For which duration should the time-proportional sampling be carried out? How much does the toxicity of the average concentration differ from the corresponding fluctuating exposure profile?

To answer these questions we carried out computer based effect modelling. We used toxicokinetic-toxicodynamic models [1, 2] for *Gammarus pulex*, *Daphnia magna* and *Pimephales promelas* as well as population models for different species of lemna and algae. The models were calibrated using toxicity test data and all models included processes which allowed effects to recover during periods of lower exposure concentrations. This type of modelling allows modelling the time course of toxicity with better accuracy than using average concentrations. The toxicity of an average concentration only equals the toxicity of the corresponding fluctuating concentration when the organism's toxicokinetics and toxicodynamics are much faster than the time-scale of the exposure fluctuations [3, 4]. This situation is called Haber's law or time effect reciprocity. At the population level the same principle holds if population dynamics are much faster than the fluctuations of the exposure profile.

We assume that the effect models used here represent the real time course of toxicity with sufficient accuracy. Based on that, we simulate the toxic effects of the fluctuating exposure profile and its corresponding average concentration and compare the two. This answers the question of how much the toxicity of the average concentration (representing time-proportional sampling) differs from the real toxicity resulting from fluctuating concentrations (based on real monitoring data from Swiss water bodies). In other words: We provide an evaluation of time-proportional water pollution sampling by approximating the systematic error due to divergence from Haber's law. By doing these simulations and comparisons for different durations (3d and 14d) we can also assess the consequences of shorter compared to longer sampling durations.

Materials and methods

Detailed reports on modelling work

The modelling work is described in more detail in three reports produced by Ibacon GmbH. These are appendices A (*G.pulex* & *P.promelas*), B (*D.magna*) & C (Lemna spec. & algae).

Monitoring data

The monitoring data was provided by the Oekotoxzentrum and derives from a monitoring campaign undertaken in 2015 in five small water bodies (Table 1) situated in intensively used agricultural areas in Switzerland. Details on the water bodies and the monitoring can be found elsewhere [5, 6]. In short, samples were taken from March to August 2015, automated samplers took half-day time-proportional composite samples. To reduce the effort of chemical analysis, samples during periods of dry weather were combined before analysis assuming that concentration peaks are rainfall driven. This results in samples with a maximum temporal resolution of 12 hours. Chemical analysis was performed by LC-MS (HRMS/MS, Orbitrap-Technology) after enrichment via online SPE.

Table 1: Description of the sampling sites

Name of water body	Swiss canton	Catchment size (km^2)
Canale Piano di Magadino	Ticino	9.0
Eschelisbach	Thurgau	2.0
La Tsatonire	Valais	2.4
Mooskanal	Berne	3.4
Weierbach	Basel Landschaft	1.6

Margin of Safety (MoS)

When simulating the toxicity of exposure profiles from monitoring data the resulting effects are often 0%, when the concentrations are below the toxic range, or 100% when the concentrations are above the toxic range. This is because real concentrations in water bodies vary across several orders of magnitude. Nothing can be learned about the questions asked here from such simulation results because we need to assess the differences in toxicity when comparing average vs. fluctuating concentrations. To overcome this problem we use the margin of safety (MoS) concept [7]. This is built on the idea that every concentration profile can be multiplied with a factor, the margin of safety, to bring it into the toxic range. This factor is larger than one if the concentration profile was originally below toxic levels or it can be smaller than one if the original concentrations were higher than the toxic range. Here we define the MoS as the factor that, when used to multiply the exposure concentrations with, results in 50% effects at the end of the simulated exposure profile. For example in simulations where the end point is survival (mortality, acute toxicity) this would be the factor to multiply concentrations in a monitoring time series with, so that after simulating 14d of exposure 50% of *G.pulex* (or *P.promelas*) would be dead. Similarly, we used 50% reduction in growth or reproductive output of *D.magna* as well as 50% reduction in biomass produced by lemna or algae populations. The MoS is calculated for simulations with the average concentration (representing time-proportional sampling) and simulations with the fluctuating exposure profile (representing real exposures). Then both MoS values can be compared to assess if the time-proportional sampling over- or underestimates the chemical hazard and by how much. Note that the MoS is similar to the toxicity – exposure ratios traditionally used in plant protection product's authorisation and can be interpreted the same way.

Simulated time windows

For each monitoring dataset we simulated moving time-windows of 14d or 3d duration. The MoS was calculated for the resulting effects at the end of each time window. Each sampling time is the starting point of one time-window. Therefore two time windows were created for each day, because the exposure data has a time step of 12h. For *D.magna* only the simulations with the 14d time window are discussed here because that duration is more relevant for the chronic endpoints modelled in *D.magna* than the 3d time window.

Substances studied

While the monitoring comprised more than 100 substances, the profiles of seven were selected for the effect modelling (Table 2). They cover five different chemical classes and modes of action.

Table 2: Selected substances, their modes of action and the most sensitive taxonomic groups based on the valid and relevant data compiled in the EQS dossiers by the Swiss Centre for Applied Ecotoxicology[1].

Substance	**Use**	**Chemical class**	**Mode of action** [8]	**Most sensitive taxonomic group**
Fungicide				
Carbendazim	Fungicide	Benzimidazole	Inhibition of beta-tubulin synthesis	Crustaceans and fish
Herbicides				
Diuron	Herbicide	Phenylurea	Inhibition of photosynthesis at photosystem II	Cyanobacteria and algae
Metazachlor	Herbicide	Chloroacetamide	Inhibition of very long chain fatty acids	Green algae and higher plants
Insecticides				
Chlorpyrifos	Insecticide	Organophosphate	Inhibition of cholinesterase	Crustaceans and insects
Diazinon	Insecticide	Organophosphate	Inhibition of cholinesterase	Crustaceans
Dimethoate	Insecticide	Organophosphate	Inhibition of cholinesterase	Insects and crustaceans
Imidachloprid	Insecticide	Neonicotinoid	Binding to postsynaptic nicotinoid receptors in the insect central nervous system (antagonist)	Insects and crustaceans

[1] See: http://www.ecotoxcentre.ch/expert-service/quality-standards/proposals-for-acute-and-chronic-quality-standards/?_ga=2.257673993.382797651.1502102030-1837988877.1458219369

Table 3: Overview on the organism, exposure profile and substance combinations. Modelled combinations are marked in grey.

Substance	Canale Piano di Magadino		Eschelisbach			La Tsatonire			Mooskanal		Weierbach		
***Gammarus pulex* (1) & *Pimephales promelas* (2) mortality**													
Carbendazim			1			1					1		
Chlorpyrifos			1	2		1	2						
Diazinon			1	2							1	2	
Dimethoate						1					1		
***Gammarus pulex* (1) & *Pimephales promelas* mortality (2), *Daphnia magna* reproduction (3)**													
Imidachloprid			1		3	1		3			1		3
Lemna (4) & algae growth (5)													
Diuron			4	5		4	5				4	5	
Metazachlor	4	5							4	5	4	5	

Endpoint mortality in *Gammarus pulex* and *Pimephales promelas*

For *G.pulex* and *P.promelas* we simulated toxic effects on survival using the toxicokinetic-toxicodynamic model General Unified Threshold model of Survival (GUTS)[9]. More specifically we used the limit cases GUTS scaled internal concentration (SIC) with stochastic death (SD) and individual tolerance (IT). As the model calibration to toxicity data for GUTS-SIC-IT resulted in much poorer quality of fit than for GUTS-SIC-SD in *G.pulex* we used only GUTS-SIC-SD for this species. For *P.promelas* we used GUTS-SIC-SD and GUTS-SIC-IT and averaged the percentage of time data from the two models. Five substances (carbendazim, chlorpyrifos, diazinon, dimethoate, imidacloprid) were assessed for *G.pulex* in two or three locations, depending on availability of monitoring data (Eschelisbach, La Tsatonire, Weierbach). Two substances were assessed for *P.promelas* in two locations (chlorpyrifos in Eschelisbach and La Tsatonire; diazinon in Eschelisbach and Weierbach). Two durations of the time window (3d and 14d) were assessed. The toxicity data for calibration of GUTS for *G.pulex* originated from previously published studies (chlorpyrifos [10], diazinon [11], imidacloprid [12]) and unpublished experiments (carbendazim, dimethoate). The toxicity data for calibration of GUTS for *P.promelas* also originated from the scientific literature [13, 14]. See more details on the GUTS model calibration and use in Appendix A.

Endpoints growth and reproduction in *Daphnia magna*

For *D.magna* we simulated effects of imidacloprid on two sub-lethal endpoints: organism growth and reproductive output (offspring) using a Dynamic Energy Budget model with toxicity module (DEBtox) [15]. The model was calibrated based on published data [16] and we simulated moving 14d time windows in three locations (Eschelisbach, La Tsatonire and Weierbach). See more details on the DEBtox model parameterisation and simulations in Appendix B.

Endpoint population biomass in duckweed and algae

To assess herbicides we simulated effects on biomass growth for metazachlor and *Lemna gibba*, diuron and *Lemna minor*, metazachlor and *Scenedesmus subspicatus* as well as diuron and the blue-green alga *Synechococcus sp*. Simulations for metazachlor were using exposure data from Mooskanal, La Tsatonire and Weierbach, whereas simulations for diuron used exposure data from Eschelisbach, La Tsatonire and Weierbach. The effect model was a simple exponential population model for biomass growth as is also used to analyse standard toxicity tests with algae and lemna. We assessed 3d and 14d moving time windows.

The simulations were based on published growth rate data. For metazachlor the data were taken from the draft assessment report. The 7d growth rates for *Lemna gibba* derive from Scheerbaum [17]. The 3 day growth data for the alga *Scenedesmus subspicatus* derive from Scheerbaum [18]. The 3 d growth rates for diuron and *Lemna minor* were published by Drost [19]. And the 3 day growth rates for the blue-green alga *Synechococcus sp* were taken from a study published by Devilla et al. [20].

Results and discussion

By how much does time-proportional sampling over- or underestimate hazard?

To answer this question we analysed the MoS values from the most toxic time windows, i.e. those with the smallest MoS values. The most toxic time window varied depending on sampling site, compound and species (see Appendices A, B and C).

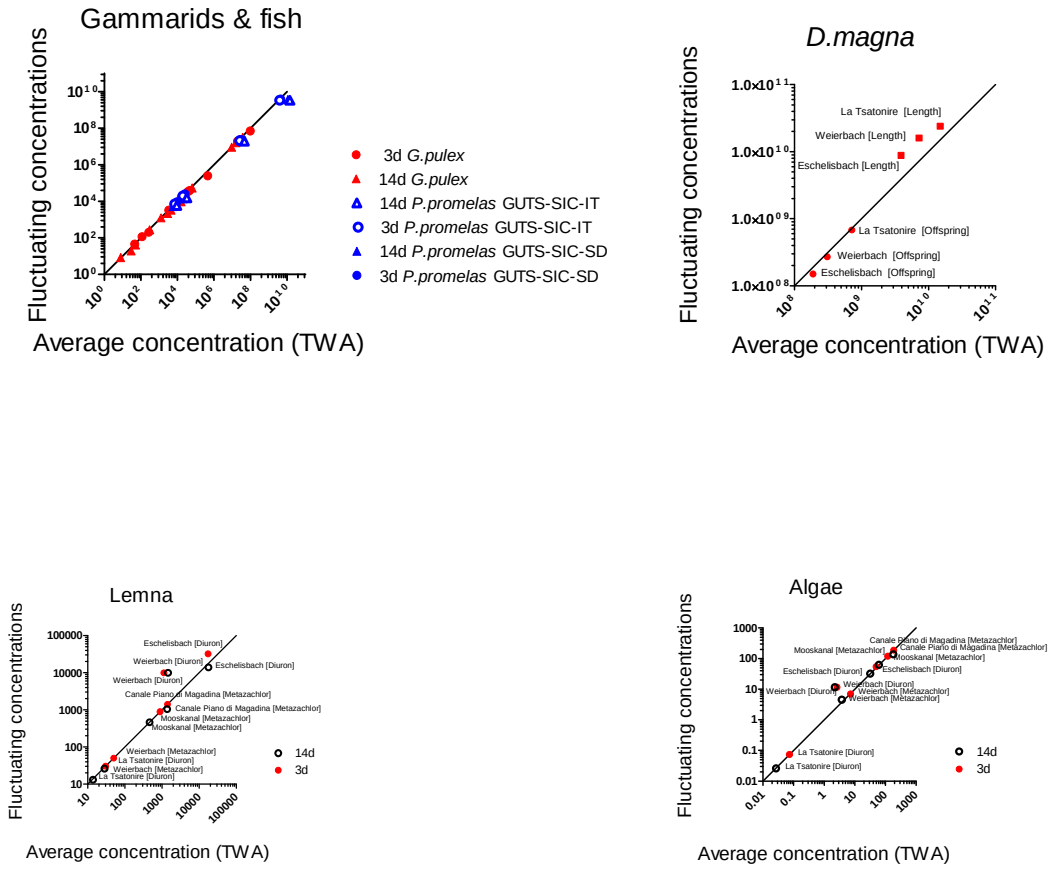

Figure 1: Comparison of margin of safety values based on time-weighted average concentrations (TWA, as proxy for time-proportional sampling) and based on fluctuating concentrations.

Generally TWA and fluctuating concentration based MoS values correlate well across orders of magnitude (Figure 1). For *G.pulex* and *P.promelas* we observe that during the most toxic exposure periods the TWA method generally predicts higher MoS values, i.e. it underestimates hazard. On average the MoS is a factor of 1.4 higher with the TWA method during the most toxic periods, with the difference being more pronounced for 14d (factor 1.7 higher) than for 3d (factor 1.1 higher). This means a time proportional sampling strategy would underestimate the hazard by a factor 1.4 on average (factor 1.7 when sampling 14d).

For *D.magna* we observe that during the most toxic exposure periods the TWA method predicts higher MoS values for offspring but lower MoS values for length, i.e. it underestimates hazard for *D.magna* offspring but overestimates hazard for *D.magna* length. On average the MoS calculated with the TWA method for length is 0.51 times the value calculated for fluctuating exposure. For effects on offspring the TWA method calculates 1.1 times the value of the fluctuating exposure (14d time window). Hence time-proportional sampling is overprotective for effects on growth and slightly under-protective for effects on offspring.

For *Lemna spec.* and algae we observe that the MoS values calculated with the TWA method are mostly equal to those calculated with the fluctuating concentrations and sometimes the TWA method overestimates hazard (i.e. it errs on the conservative side). On average the MoS is a factor of 0.8 lower with the TWA method during the most toxic periods for *Lemna spec*. with the 3d time window and equal with the fluctuating concentrations for the 14d time window. For algae the MoS is a factor of 0.9 lower on average with the TWA method during the most toxic periods with the 3d and also the 14d time window.

In summary, these findings suggest that time-proportional sampling would underestimate hazard for gammarids, daphnids and fish and slightly overestimate hazards for duckweed and algae. However the hazards, here approximated as MoS values, range over twelve orders of magnitude, whilst the differences between the time-proportional sampling method and the real, fluctuating exposure are comparatively small. In other words, a difference in the MoS value by a factor four or less is very small when MoS values range from 0.027 to 2.4×10^{10}. As the MoS value is a proxy for hazard our analysis shows that in the datasets provided the hazard in small Swiss streams varies over twelve orders of magnitude depending on location, compound and species. In this context an error of factor four or less due to the time-proportional sampling method is very small, especially when considering that any alternative method is also associated with error.

How often does time-proportional sampling over- or underestimate hazard?

To answer this question we analysed the MoS values from all time-windows over the whole length of the monitoring profiles.

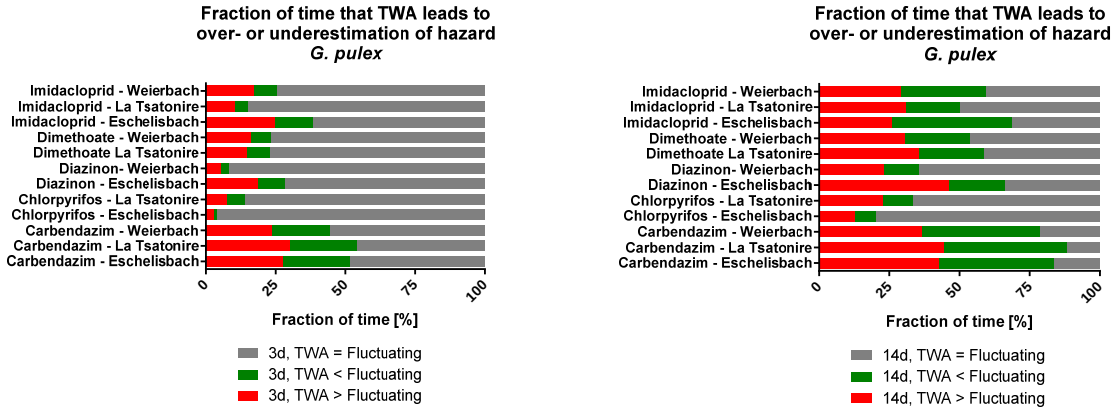

Figure 2: Fraction of time windows during which time-proportional sampling (TWA) would over- or underestimate the margin of safety for gammarids. TWA > fluctuating means the hazard is underestimated and TWA < fluctuating means that time-proportional sampling would err on the conservative side. Comparison of 3d (left) vs 14d (right).

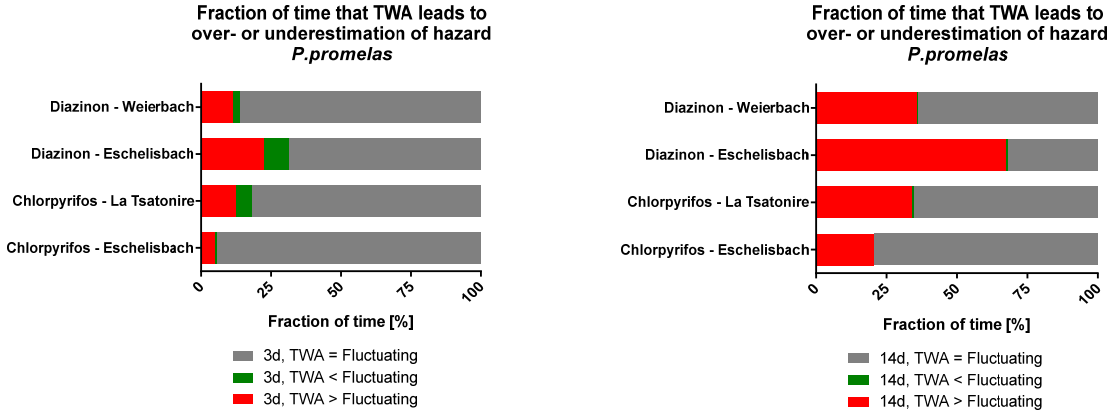

Figure 3: Fraction of time windows during which time-proportional sampling (TWA) would over- or underestimate the margin of safety for fish. TWA > fluctuating means the hazard is underestimated and TWA < fluctuating means that time-proportional sampling would err on the conservative side. Comparison of 3d (left) vs 14d (right).

The simulations with gammarids and fish show that during a 14d period the TWA method deviates more often from the fluctuating exposure assessment than during a 3d window (Figure 2, 3). Both, the 3d duration and the 14d duration simulations, result in the time-proportional sampling erring more often on the risky side for *G.pulex* (17% and 32% of time, respectively) than on the conservative side (11% and 26% of time, respectively). For *P.promelas* the 3d simulations err 13% of the time on the risky side and 4% of time on the conservative side, and the 14d simulations err 39% of time on the risky side and 0% of time on the conservative side. This is because peak exposures are driving the toxicity for diazinon and chlorpyrifos in *P.promelas*. Averaging out the peaks to lower TWA concentrations almost always reduces the toxicity for these two compounds in *P.promelas*, however the absolute error is a factor of 2.1 or less (see MoS values, above).

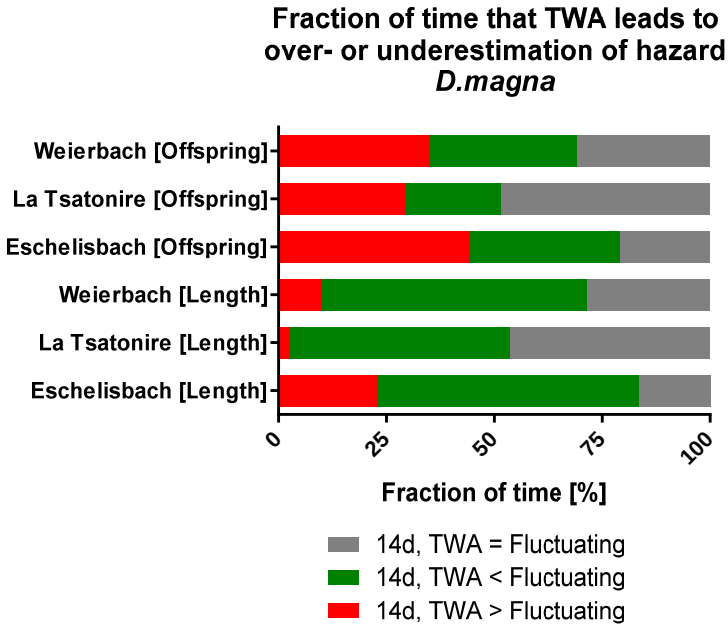

Figure 4: Fraction of time windows during which time-proportional sampling (TWA) would over- or underestimate the margin of safety for *D.magna*. TWA > fluctuating means the hazard is underestimated and TWA < fluctuating means that time-proportional sampling would err on the conservative side. Comparison of 3d (left) vs 14d (right).

The simulations with *D.magna* and imidacloprid show that over- and underestimations of hazard are frequent (Figure 4). Overestimation of hazard in the time-proportional sampling occurs more frequently overall (44% of time overestimated vs. 24% underestimated). This is due overestimation of hazard for effects on growth (58% of time overestimated vs. 12% underestimated), whereas effects on reproduction are generally underestimated by the time-proportional sampling (30% of time overestimated vs. 36% underestimated).

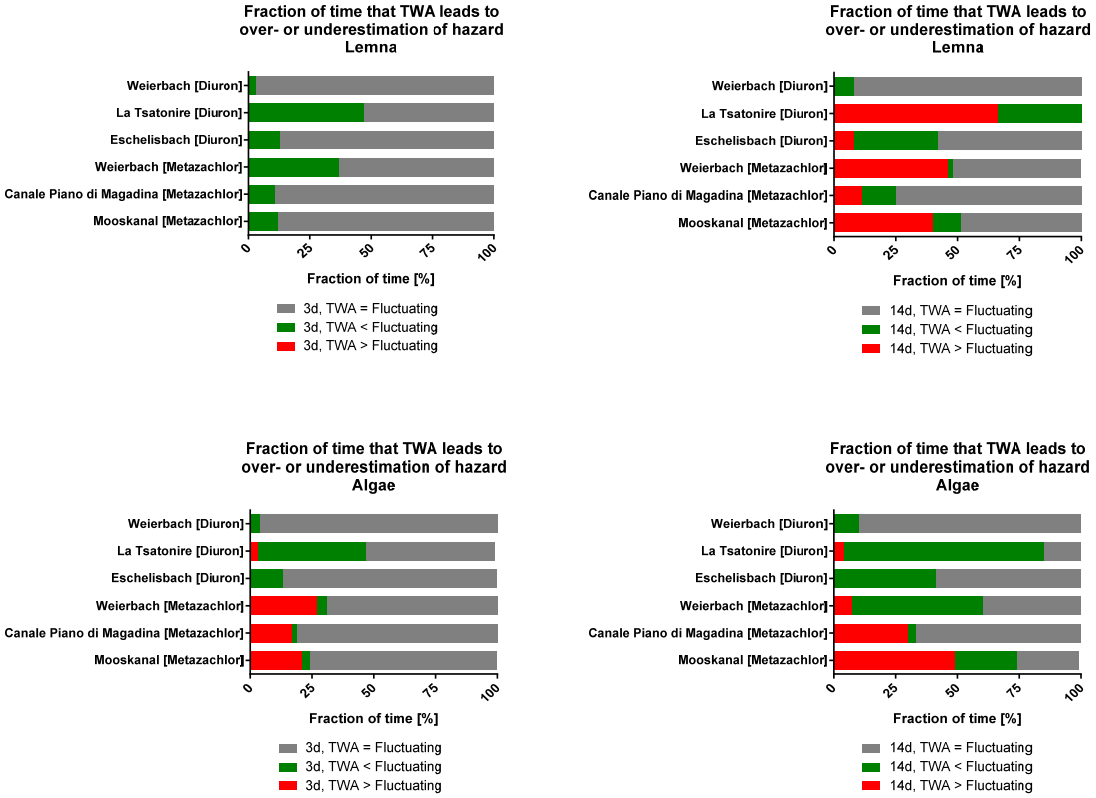

Figure 5: Fraction of time windows during which time-proportional sampling (TWA) would over- or underestimate the margin of safety for duckweed and algae. TWA > fluctuating means the hazard is underestimated and TWA < fluctuating means that time-proportional sampling would err on the conservative side. Comparison of 3d (left) vs 14d (right).

Simulations of biomass growth with duckweed and algae show that during a 14d period the TWA method deviates more often from the fluctuating exposure assessment than during a 3d window (Figure 5). For duckweed (*Lemna spec.*) we observe that the 3d simulations overestimate hazard (i.e. they err on the conservative side), but the 14d simulations seems to underestimate hazard (28% of time) more often than overestimating it (17% of time). For algae we observe that it depends more strongly on the location and substance sampled whether the 3d or 14d duration over- or underestimates hazard compared to the other species. However the time-proportional sampling would generally overestimate hazard for algae on average (overestimation: 12% (3d) and 36% (14d); underestimation: 11% (3d) and 15% (14d)).

Conclusions

Generally the model simulations show that a longer sampling period (14d) for time-proportional water sampling leads to more deviations from the real hazard than shorter sampling durations (3d). However the overall picture shows that under- and overestimations of hazard occur with

approximate equal frequency, although for some locations, compounds and species we observed clear under- or overestimation of hazard (e.g. 14d fish and 3d lemna, respectively). When under- or over-estimations of hazard occur, then they are very small compared to the overall variation in hazard observed. In this dataset, the hazard to aquatic organisms from pesticides in Swiss streams ranged over twelve orders of magnitude, from 0.027 to 2.4×10^{10} (where small values indicate a hazard and large values indicate safety). We found that the time-proportional sampling method is associated with an error of factor four or less in the hazard value towards the risky side and an error of factor nine towards the conservative side. This error margin is very small compared to twelve orders of variation in hazard across locations, compounds and species.

When using a 3d sampling duration our simulations show considerably fewer time periods when time-proportional sampling either under- or overestimates hazard compared to 14d sampling duration. Therefore it is reasonable to use a 3d sampling duration, or a similarly short sampling duration, for assessment of acute effects. For chronic effects assessment a longer sampling duration is more realistic. With the exception of fish, the 14d sampling duration simulated here shows a balanced frequency of over- or underestimations of hazard and a small overall error compared to the magnitude of variation in hazard. Hence using 14d time-proportional sampling to measure micropollutants for assessment of chronic effects appears reasonable from the perspective of time-variable exposure, but more simulations with different compounds would be desirable to strengthen the evidence base, especially in case of fish.

It is important to bear in mind that only a small selection of sub-lethal effects were simulated in a very small number of species and locations. Other effects, such as those on the endocrine system, epigenetic effects or behavioural changes might be ecologically relevant for chronic risk assessment of micropollutants, as well as mixture toxicity and interactions with other, non-chemical stressors. However these aspects were not considered here.

Appendices

Appendix A: R.Kuhl & E.Zimmer (2017): Simulation based assessment of Haber's law using modelling approaches (TKTD, GUTS) - *Gammarus pulex* and *Pimephales promelas*. Ibacon GmbH study number: 120661253.

Appendix B: R.Kuhl & E.Zimmer (2017): Simulation based assessment of Haber's law using modelling approaches (TKTD, GUTS) – *Daphnia magna*. Ibacon GmbH study number: 120661253.

Appendix C: R.Kuhl & E.Zimmer (2017): Simulation based assessment of Haber's law using modelling approaches (TKTD, GUTS) – Algae and Lemna. Ibacon GmbH study number: 120661253.

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Final Report

Simulation based assessment of Haber's law using modelling approaches (TKTD, GUTS)

- ***Gammarus pulex*** **and** ***Pimephales promelas***

Author: Roland Kuhl
Dr. Elke Zimmer

Study Completion Date: August 18, 2017

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Study No. 120661253

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1. Survey of the Study

1.1 General Information

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Ibacon Study No.: 120661253

Project Staff:

Test Facility Management: Dr. Melanie Lichtenberger

Study Director: Roland Kuhl

1.2 Archiving

The following documents and materials will be archived for the at least 3 years:

- All electronic raw data
- the electronic final report
- any electronic final report amendment or any revised final report

Following the date on which the final report at

ibacon GmbH
Germany

After the archiving period, all raw data or material relating to the study will be discarded without the Sponsor's prior written consent.

1.3 Final Report Approval

Dr. Ecke Zimmer
für

Study Director: Roland Kuhl

i.A. E. Zimmer

date: 31.08.2017

Test Facility Management: Dr. Melanie Lichtenberger

date: August 31, 2017

2. Materials and Methods

2.1 Definitions

Margin of Safety: The margin of safety is the value witch which a concentration is multiplied to lead to 50% mortality in the simulated population.

Time window: A 3 day or 14 day timespan for which the time weighted average of all measured concentrations is calculated. Since two samplings were performed each day, two time windows are calculated to start each day. For the 3 day simulations, 211 time windows were calculated, while 200 time windows were calculated for the 14 day simulations.

Underestimation: A margin of safety is considered as underestimation if the calculated margin for a specific day is calculated to be lower than the respective margin of safety from the real exposure profile.

Survival: Survival is calculated by $\Delta S = -(h + hb) * S$

2.2 Model equations

Change in internal concentration: dCi = kd * (K * c - Ci)

Hazard rate: h = bw * max(0,Ci-mw)

Change in Survival probability: dS = -(h + hb)* S

Background hazard rate: dS = -hb* S

Survival: ΔS=-(h+hb)*S

2.3 GUTS parameters:

kd: Dominant rate constant (d-1)

mw: Median threshold for survival (nmol/L)

hb: Background hazard rate (1/d)

bw: Killing rate (L/nmol/d)

Fs: Fraction spread of NEC distribution

2.4 Parameter Estimation and Simulations for *Gammarus pulex* and *Pimephales Promelas*

Test Items:	Carbendazim, Chlorpyrifos, Diazinon, Dimethoate, Imidacloprid	
Test Species:	*Gammarus pulex*, *Pimephales promelas*	
Software:	Matlab R2016a 64-bit version 9.0.0.341360 (February 11, 2016) Matlab 2017a 64-bit version 9.2.0.556.344 (March 27, 2017) BYOM for Matlab version 4.01 (May 12, 2017) GUTS version 2.0 (April 27, 2017)	
Modell Parameters:	Solver: ode45	
Parameter Estimation Settings:	Relative Tolerance:	$1x10^{-4}$
	Absolute Tolerance:	$1x10^{-7}$
	Initial Step Size:	Max(t)/1000
	Maximum Step Size:	Max(t)/100
	where t is the time vector of the data	

Parameter Estimation:

At first, the parameter hb (background hazard rate) was fitted to the control values of all tests present for each respective test Item. Afterwards, the parameters kd (dominant rate constant), mw (median threshold for survival) and bw (killing rate) in case of stochastic death (SD) respectively Fs (fraction spread of NEC distribution) for individual tolerance (IT) were fitted.

The 95% confidence intervals for the parameters were calculated.

With the obtained parameters, simulations were run using different time windows for a measured exposure profile from the three locations of Tsatonire, Eschelisbach and Weierbach. The simulations were run for the normal profile with two measurements on each day. To compare different sampling regimes, the time weighted average (TWA) of each exposure profile was calculated for 3 day windows and for 14 day windows.

For these different concentrations profiles, the margins of safety (MoS) that lead to 50% mortality at the end of the time window were calculated and compared. As a maximum safety margin, we assumed $1x10^{19}$.

3. Results for *Gammarus pulex* and *Pimephales promelas*

Time Windows:

Time windows were calculated beginning from each sampling time. Since sampling took place twice a day, two 3 day and 14 day time windows start each day, respectively.

Simulation Results:

The graphs show the safety margin as $1x10^{19}$ when either the concentration was zero, or when the concentration times the safety factor was still lower that the no-effect concentration.

Gammarus pulex:

The simulations for *Gammarus pulex* show no mortality at the end of the full exposure period for all chemical in all locations except for Carbendazim in the Weierbach. There a slight mortality was observed after a peak in the exposure profile (see Figure 8).

In case of the *Gammarus pulex* simulations, the simulations carried out with the 3 day TWA windows underestimated the real exposure (Margin of safety for TWA > pulse) in 337 cases, while the 14 day TWA calculations underestimated the real exposure in 544 cases.

Overestimation occurred in 371 time windows for the 3d TWA simulations and for 803 time windows in the 14d TWA simulations.

Pimephales promelas:

The simulations for *Pimephales promelas* showed that no mortality would occur for all scenarios.

Table 1. The 3 most toxic time windows leading to the lowest margins of safety for *Gammarus pulex* using GUTS-SIC-SD

	Lowest Margin of Safety for GUTS-SIC-SD											
Chemical	3d TWA						3d pulse					
and Location	1st	d	2nd	d	3rd	d	1st	d	2nd	d	3rd	d
Carbendazim												
Eschelisbach	10418	70	11235	69	13309	71	8576	70	8576	71	8576	72
La Tsatonire	16023	110	16023	111	16023	112	16023	110	16023	111	16023	112
Weierbach	3341	138	3859	137,5	3859	139	3341	138	3341	139	3341	142
Chlorpyrifos												
Eschelisbach	117	78	117	79	117	80	117	78	117	79	117	80
La Tsatonire	46,365	151,5	50	150,5	50	152	46,365	151,5	46,365	152	46,365	152,5
Diazinon												
Eschelisbach	271	16,5	322	17	324	15,5	203	17	203	18	335	16,5
Weierbach	46794	148	55788	148,5	70016	147	39735	148,5	39735	149,5	72242	148
Dimethoate												
La Tsatonire	445940	123	445940	124	521650	125	250210	60	445940	124	445940	125
Weierbach	36946	138	38728	137	40217	139	33406	138	33406	139	38728	142
Imidacloprid												
Eschelisbach	1,9E+07	56	1,9E+07	57	2,3E+07	58	1,9E+07	56	1,9E+07	57	1,9E+07	58
La Tsatonire	9,6E+07	141	1,0E+08	142	1,2E+08	140	7,1E+07	142	7,1E+07	143	7,1E+07	147
Weierbach	2,8E+07	29,5	3,0E+07	58,5	3,1E+07	28	2,3E+07	58,5	2,3E+07	59	2,8E+07	30
	min: 46 max: 1.2E+08 mean: 1.3E+07						min: 46 max: 7.1E+07 mean: 9.6E+06					

d: Day at which the respective time windows starts

Table 2. The 3 most toxic time windows leading to the lowest margins of safety for *Gammarus pulex* using GUTS-SIC-SD

	Lowest Margin of Safety for GUTS-SIC-SD											
Chemical	14d TWA						14d pulse					
and Location	1st	d	2nd	d	3rd	d	1st	d	2nd	d	3rd	d
Carbendazim												
Eschelisbach	1273	61,5	1305	62	1322	60	1257	63	1257	63,5	1273	64,5
La Tsatonire	2872	105	2872	105,5	2909	106,5	2171	109	2171	110	2282 .6	108
Weierbach	302	137,5	303	137	309	138	276	137,5	282	138	292	137
Chlorpyrifos												
Eschelisbach	29,5	70	29,5	71	29,5	72	19,3	77	20,0	76	20,3	75
La Tsatonire	7,9	143	8,0	144	8,3	142	8,4	144	8,4	148	8,4	148,5
Diazinon												
Eschelisbach	50	13,5	50	14,5	50	15,5	41	16,5	43	15,5	45	14,5
Weierbach	15688	138,5	15688	139,5	15688	140,5	9459	148	9947	147,5	10329	146,5
Dimethoate												
La Tsatonire	64817	119	64817	120	64817	121	54032	121	54032	121,5	54032	122
Weierbach	4420	137	4420	137,5	4476	135,5	3341	137,5	4007	138	4048	137
Imidacloprid												
Eschelisbach	9,1E+06	48,5	9,1E+06	49,5	9,1E+06	50,5	9,1E+06	48,5	9,1E+06	49,5	9,1E+06	50,5
La Tsatonire	3,7E+07	141	3,8E+07	140,5	3,9E+07	139,5	3,1E+07	141	3,6E+07	140,5	3,7E+07	139,5
Weierbach	1,6E+07	21	1,6E+07	22	1,6E+07	22,5	1,6E+07	22	1,6E+07	22,5	1,6E+07	23
	min: 8 max: 3.9E+07 mean: 5.3E+06						min: 8 max: 3.7E+07 mean: 5.1E+06					

d: Day at which the respective time windows starts

The timeframes shown here are the ones in which the TWA simulation resulted in a lower margin of safety than the simulation using the exact exposure profile.

Table 3. The 3 most toxic time windows leading to the lowest margins of safety for *Pimephales promelas* using GUTS-SIC-SD

Chemical and Location	Lowest Margin of Safety for GUTS-SIC-SD											
	3d TWA						3d pulse					
	1st	d	2nd	d	3rd	d	1st	d	2nd	d	3rd	d
Chlorpyrifos												
Eschelisbach	25927	77,5	25927	78,5	25927	79,5	25927	77,5	25927	78,5	25927	79,5
La Tsatonire	9378	151,5	10502	150,5	10502	152	9378	151,5	9617	152	9862	150,5
Diazinon												
Eschelisbach	2,9E+07	16,5	3,4E+07	17	3,4E+07	15,5	2,3E+07	16,5	2,8E+07	17	3,0E+07	15,5
Weierbach	4,4E+09	148	5,3E+09	148,5	6,6E+09	147,5	3,9E+09	148	4,4E+09	148,5	5,4E+09	147,5
	min: 9378 max: 6.6E+09 mean: 1.4E+09						min: 9378 max: 5.4E+09 mean: 1.2E+09					

d: Day at which the respective time windows starts

Table 4. The 3 most toxic time windows leading to the lowest margins of safety for *Pimephales promelas* using GUTS-SIC-SD and Number of underestimated timeframes

Chemical and Location	Lowest Margin of Safety for GUTS-SIC-SD											
	14d TWA						14d pulse					
	1st	d	2nd	d	3rd	d	1st	d	2nd	d	3rd	d
Chlorpyrifos												
Eschelisbach	20065	71	20065	72	20065	72,5	14008	71	14008	72	14008	72,5
La Tsatonire	5662	143	5723	144	6019	142	5162	143	5227	144	5533	144,5
Diazinon												
Eschelisbach	3,5E+07	15	3,5E+07	15,5	3,6E+07	14	2,3E+07	6,5	2,3E+07	16,5	2,3E+07	7
Weierbach	1,1E+10	163	1,1E+10	164	1,1E+10	165	3,7E+09	174	3,8E+09	164	3,8E+09	165
	min: 5652 max: 1.1E+10 mean: 2.6E+09						min: 5162 max: 3.8E+09 mean: 9.5E+08					

d: Day at which the respective time windows starts

The timeframes shown here are the ones in which the TWA simulation resulted in a lower margin of safety than the simulation using the exact exposure profile.

Table 5. The 3 most toxic time windows leading to the lowest margins of safety for *Pimephales promelas* using GUTS-SIC-IT

Chemical and Location	Lowest Margin of Safety for GUTS-SIC-IT 3d TWA 1st	d	2nd	d	3rd	d	3d pulse 1st	d	2nd	d	3rd	d
Chlorpyrifos												
Eschelisbach	19766	77,5	19766	78,5	19766	79,5	19766	77,5	19766	78,5	19766	79,5
La Tsatonire	6950	151,5	7875	150,5	7875	152	6950	151,5	7302	150,5	7776	152
Diazinon												
Eschelisbach	2,6E+07	16,5	3,0E+07	17	3,0E+07	15,5	2,1E+07	16,5	2,3E+07	15,5	2,6E+07	17
Weierbach	3,9E+09	148	4,7E+09	148,5	5,9E+09	148	3,4E+09	148	3,9E+09	148,5	4,3E+09	147,5
	min: 6950 max: 5.9E+09 mean: 1.2E+09						min: 6950 max: 4.3E+09 mean: 9.7E+08					

d: Day at which the respective time windows starts

Table 6. The 3 most toxic time windows leading to the lowest margins of safety for *Pimephales promelas* using GUTS-SIC-IT and Number of underestimated timeframes

Chemical and Location	Lowest Margin of Safety for GUTS-SIC-IT 14d TWA 1st	d	2nd	d	3rd	d	14d pulse 1st	d	2nd	d	3rd	d
Chlorpyrifos												
Eschelisbach	32759	71	32759	72	32759	72,5	15576	71	15576	72	15576	72,5
La Tsatonire	9152	143	9329	144	9811	142	5936	143	5936	144	5936	144,5
Diazinon												
Eschelisbach	4,6E+07	12	4,6E+07	12,5	4,6E+07	13	2,0E+07	6,5	2,0E+07	7	2,0E+07	7,5
Weierbach	1,4E+10	140	1,4E+10	140,5	1,4E+10	142	3,4E+09	140	3,4E+09	140,5	3,4E+09	141,5
	min: 9152 max: 1.4E+10 mean: 3.5E+09						min: 5936 max: 3.4E+09 mean: 8.5E+08					

d: Day at which the respective time windows starts

The timeframes shown here are the ones in which the TWA simulation resulted in a lower margin of safety than the simulation using the exact exposure profile.

Table 7. Comparison of MoS of simulation results of *Gammarus pulex* from 3d and 14d TWA calculations with pulse exposure results

Chemical and Location	Comparison of TWA simluation vs. real exposure scenario GUTS-SIC-SD 3d <		GUTS-SIC-SD 3d >		GUTS-SIC-SD 14d <		GUTS-SIC-SD 14d >	
Carbendazim								
Eschelisbach	51	24 %	58	27 %	82	41 %	85	43 %
La Tsatonire	51	24 %	63	30 %	87	44 %	89	45 %
Weierbach	44	21 %	50	24 %	84	42 %	73	37 %
Chlorpyrifos								
Eschelisbach	2	1 %	6	3 %	15	8 %	25	13 %
La Tsatonire	13	6 %	16	8 %	22	11 %	45	23 %
Diazinon								
Eschelisbach	21	10 %	39	18 %	40	20 %	92	46 %
Weierbach	6	3 %	11	5 %	25	13 %	46	23 %
Dimethoate								
La Tsatonire	17	8 %	31	15 %	46	23 %	71	36 %
Weierbach	15	7 %	34	16 %	46	23 %	61	31 %
Imidacloprid								
Eschelisbach	29	14 %	52	25 %	85	43 %	52	26 %
La Tsatonire	10	5 %	22	10 %	38	19 %	62	31 %
Weierbach	18	9 %	36	17 %	61	31 %	58	29 %

<: The margin of safety for the TWA simulation was lower that the margin of safety for the pulse simulation

>: The margin of safety for the TWA simulation was higher that the margin of safety for the pulse simulation

Table 8. Comparison of MoS of simulation results of *Pimephales promelas* from 3d and 14d TWA calculations with pulse exposure results

	Times at which the *Pimephales promelas* TWA simluation underestimates the real exposure scenario							
Chemical and Location	GUTS-SIC-SD 3d		GUTS-SIC-IT 3d		GUTS-SIC-SD 14d		GUTS-SIC-IT 14d	
	<	>	<	>	<	>	<	>
Chlorpyrifos								
Eschelisbach	2 1 %	9 4 %	0 0 %	12 6 %	0 0 %	41 21 %	0 0 %	41 21 %
La Tsatonire	16 8 %	21 10 %	8 4 %	31 15 %	2 1 %	67 34 %	0 0 %	69 35 %
Diazinon								
Eschelisbach	18 9 %	48 23 %	19 9 %	47 22 %	3 2 %	133 67 %	0 0 %	136 68 %
Weierbach	5 2 %	24 11 %	5 2 %	24 11 %	1 1 %	71 36 %	0 0 %	72 36 %

<: The margin of safety for the TWA simulation was lower that the margin of safety for the pulse simulation

>: The margin of safety for the TWA simulation was higher that the margin of safety for the pulse simulation

4. References

Jager T., Albert C., Preuss T.G., Ashauer R. (2011). General unified threshold model of survival - a toxicokinetic-toxicodynamic framework for ecotoxicology. Environmental Science & Technology 45 (7): 2529–2540. DOI: 10.1021/es103092a

5. Distribution of the Final Report

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Appendix I: Tables and Figures

Table 9. GUTS-Parameters for *Gammarus pulex* for Carbendazim and Chlorpyrifos

Parameter	Carbendazim	Confidence Intervals	Chlorpyrifos	Confidence Intervals
kd	0.3141	0.1949 - 0.4464	0.1952	0.1497 - 0.2435
mw	0.003086	0 - 10.85	0.1362	0.1157 - 0.1501
hb	0.02724	0.01724 - 0.04059	0.01073	0.006926 - 0.0157
bw	0.0002693	0.0002137 - 0.0003691	0.8874	0.6717 - 1.111

Table 10. GUTS-Parameters for *Gammarus pulex* for Diazinon and Dimethoate

Parameter	Diazinon	Confidence Intervals	Dimethoate	Confidence Intervals
kd	0.08115	0.05558 - 0.1328	0.3027	0.2055 - 0.4134
mw	4.619	3.111 - 5.921	4.657E-09	0 - 312.7
hb	0.02449	0.01599 - 0.03566	0.01748	0.009848 - 0.02823
bw	0.02355	0.0125 - 0.03631	0.000029068	2.356e-05 - 3.73e-05

Table 11. Parameters for *Gammarus pulex* and Imidacloprid

Parameter	GUTS-SIC-SD	Confidence Intervals	GUTS-SIC-IT	Confidence Intervals
kd	6.931	3.604 - 10	1.00E-05	1e-05 - 0.01328
mw	0.01843	0 - 6.346	0.06957	0.04437 - 97.01
hb	0.009548	0.005873 - 0.01447	0.009548	0.005873 - 0.01447
bw	0.0001785	0.0001472 - 0.000215	-	-
Fs	-	-	267.1	102.3 - 1000

Table 12. Parameters for *Pimephales promelas* and Chlorpyrifos

Parameter	GUTS-SIC-SD	Confidence Intervals	GUTS-SIC-IT	Confidence Intervals
kd	10	7.538 - 10	0.8943	0.743 - 1.054
mw	65.44	60.37 - 67.96	201.1	183.6 - 218.9
hb	0.000000288	0 - 0.004802	0.000000288	0 - 0.004802
bw	0.001789	0.001519 - 0.002089	-	-
Fs	-	-	2.857	2.463 - 3.432

Table 13. Parameters for *Pimephales promelas* and Diazinon

Parameter	GUTS-SIC-SD	Confidence Intervals	GUTS-SIC-IT	Confidence Intervals
kd	10	5.39 - 10	1.262	0.9073 - 1.682
mw	6102	4795 - 6590	9336	8091 - 1.073e+04
hb	0	0 - 1e+06	0	0 - 1e+06
bw	8.72E-05	5.425e-05 - 0.000128	-	-
Fs	-	-	2.252	1.8 - 3.234

Parameters and Confidence Intervals for *Gammarus pulex* and Carbendazim

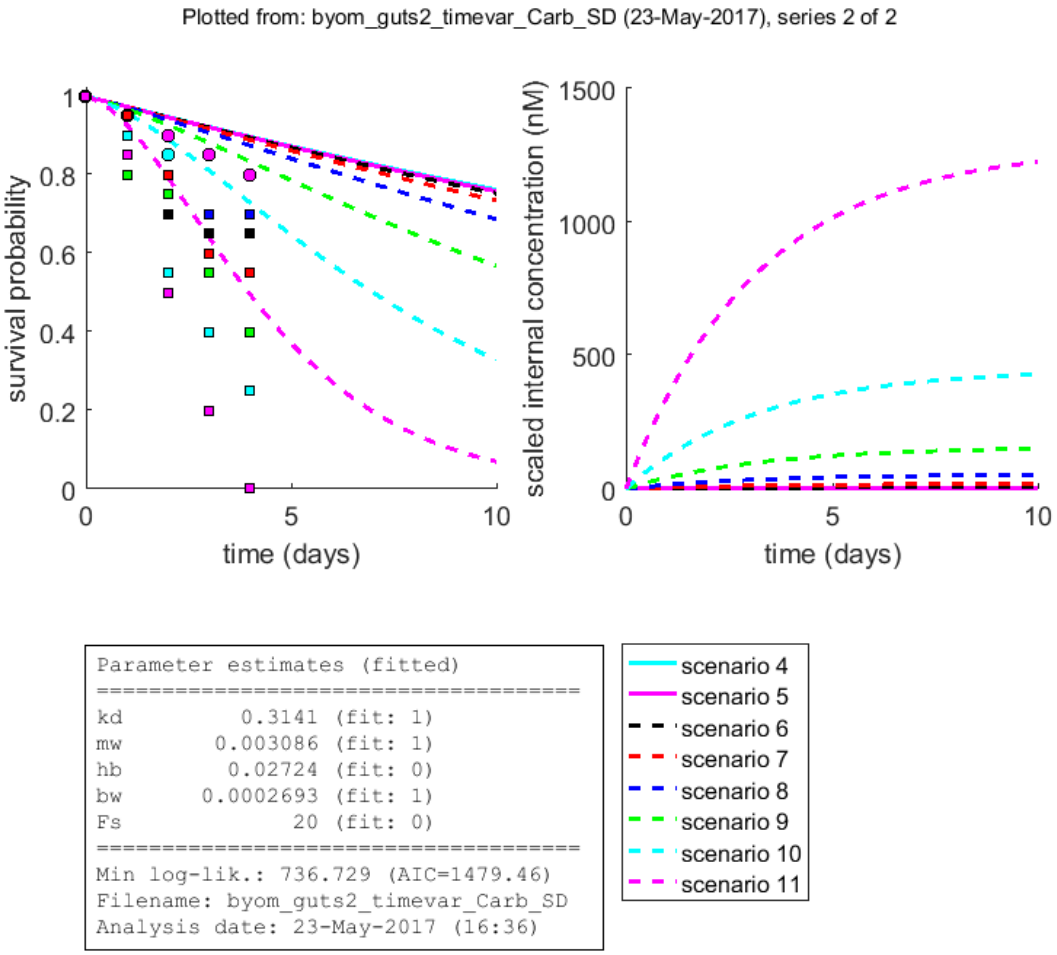


Figure 1. Graph for *Gammarus pulex* and Carbendazim estimated GUTS-SIC-SD parameters

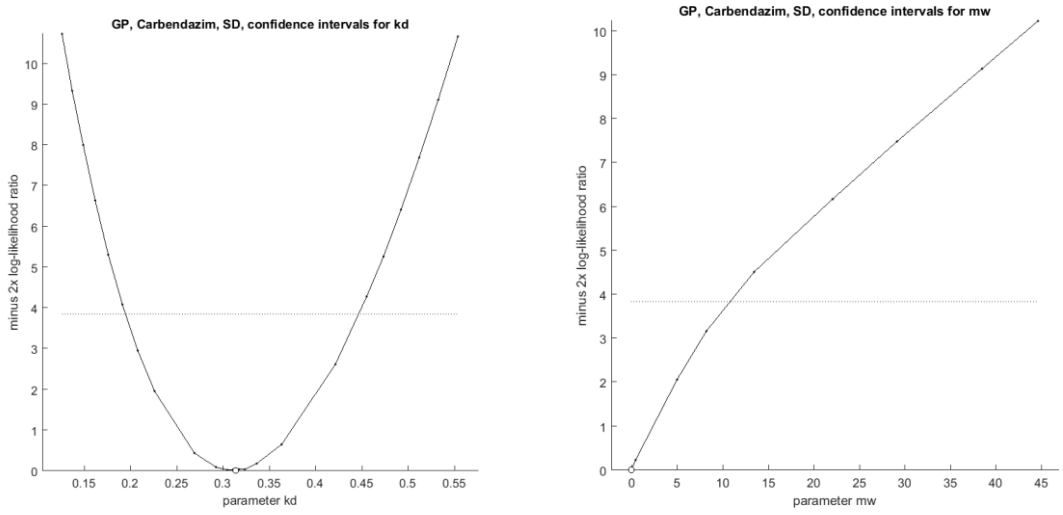


Figure 2. Confidence Intervals for *Gammarus pulex* and Carbendazim for GUTS-SIC-SD parameter kd and mw

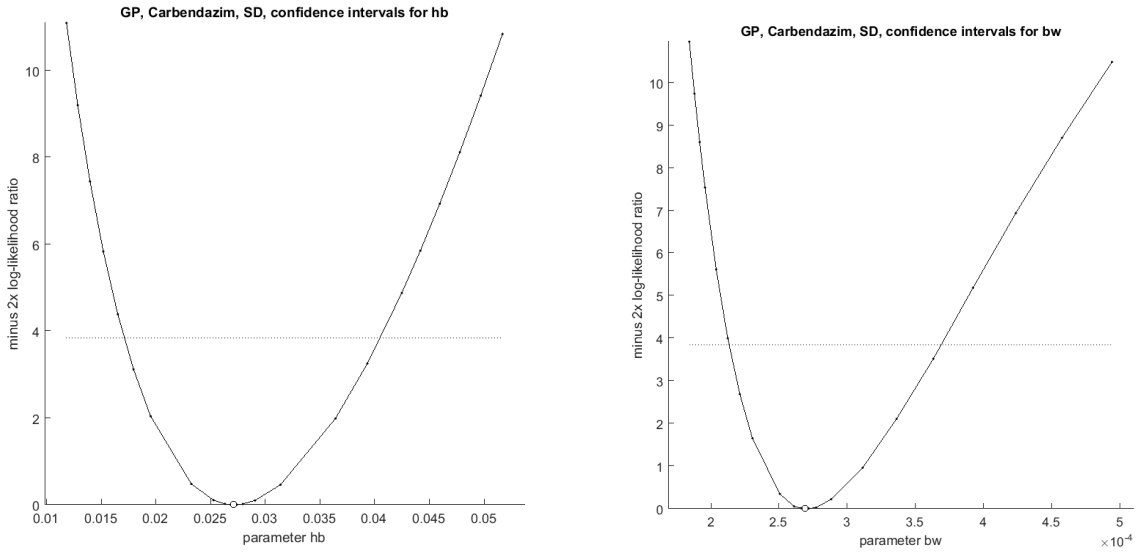


Figure 3. Confidence Intervals for *Gammarus pulex* and Carbendazim for GUTS-SIC-SD parameter hb and bw

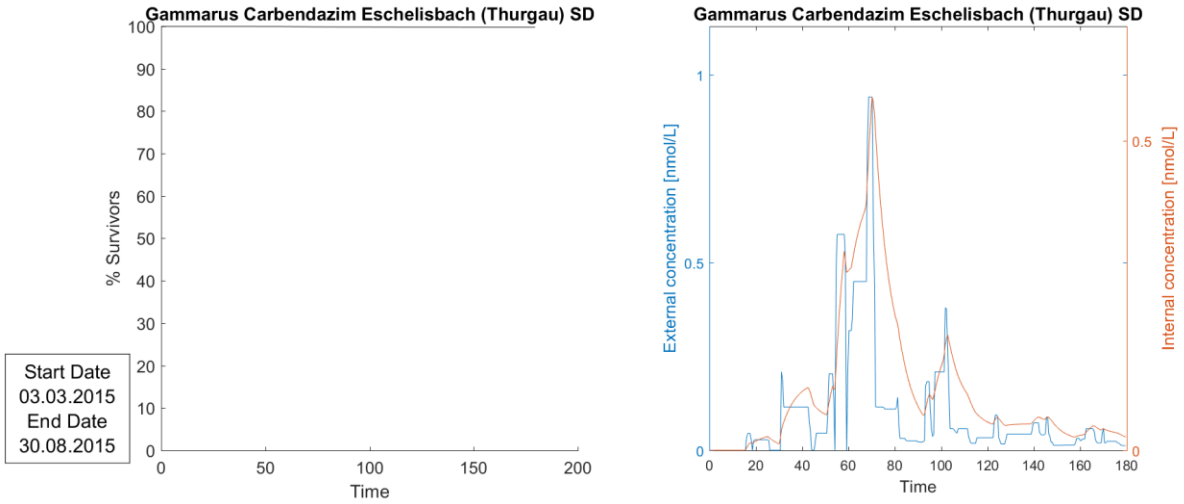


Figure 4. The simulated survival of *Gammarus pulex* for Carbendazim in Eschelisbach (Thurgau) with GUTS-SIC-SD for the full realistic exposure scenario (left) and the corresponding external and internal concentrations (right).

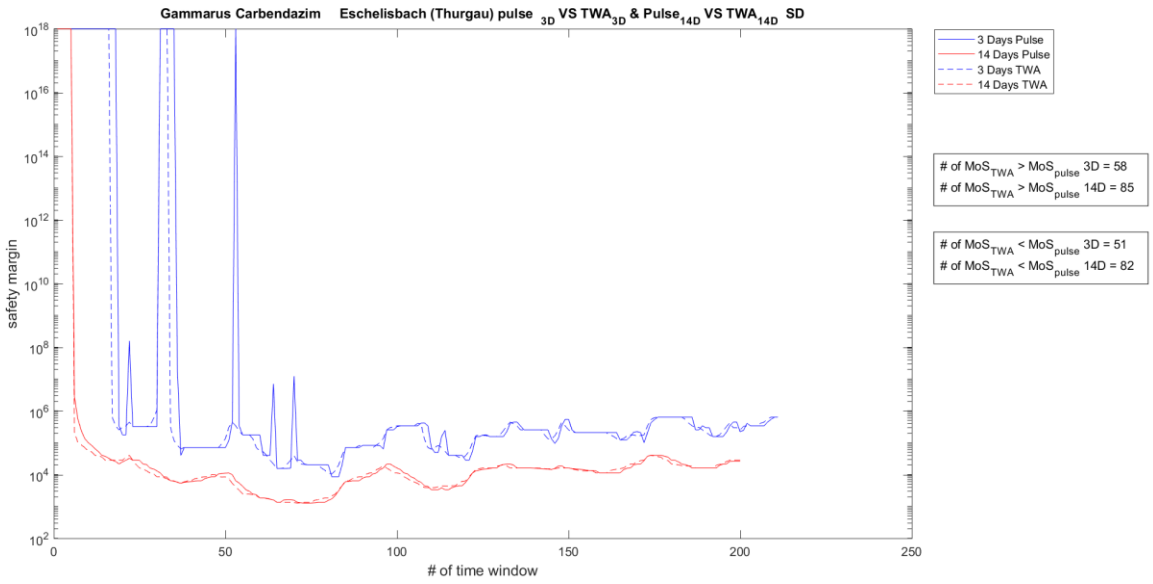


Figure 5. Margins of Safety with GUTS-SIC-SD pulse, 3 day TWA and 14 day TWA

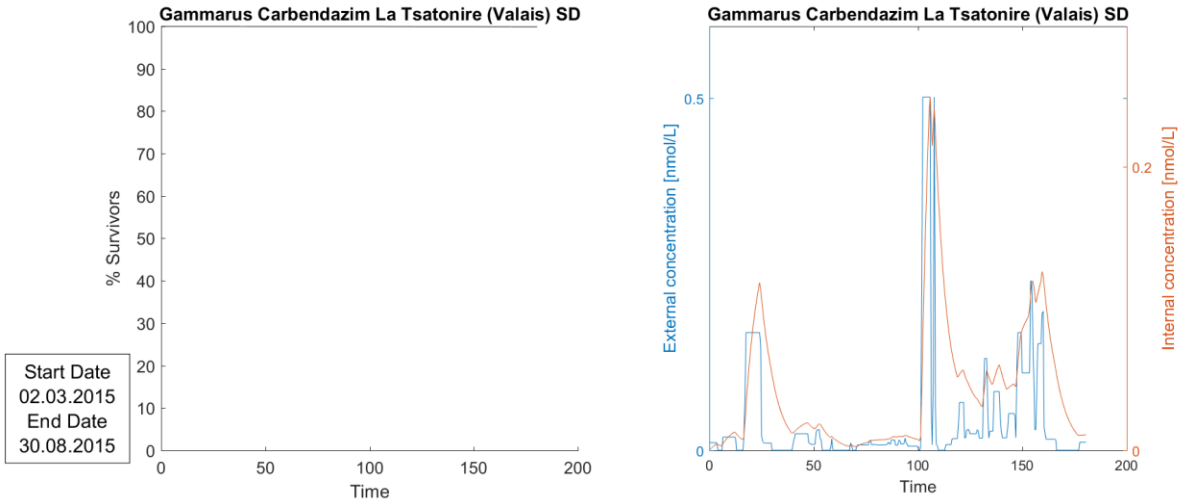


Figure 6. The simulated survival of *Gammarus pulex* for Carbendazim in La Tsatonire (Valais) with GUTS-SIC-SD for the full realistic exposure scenario (left) and the corresponding external and internal concentrations (right).

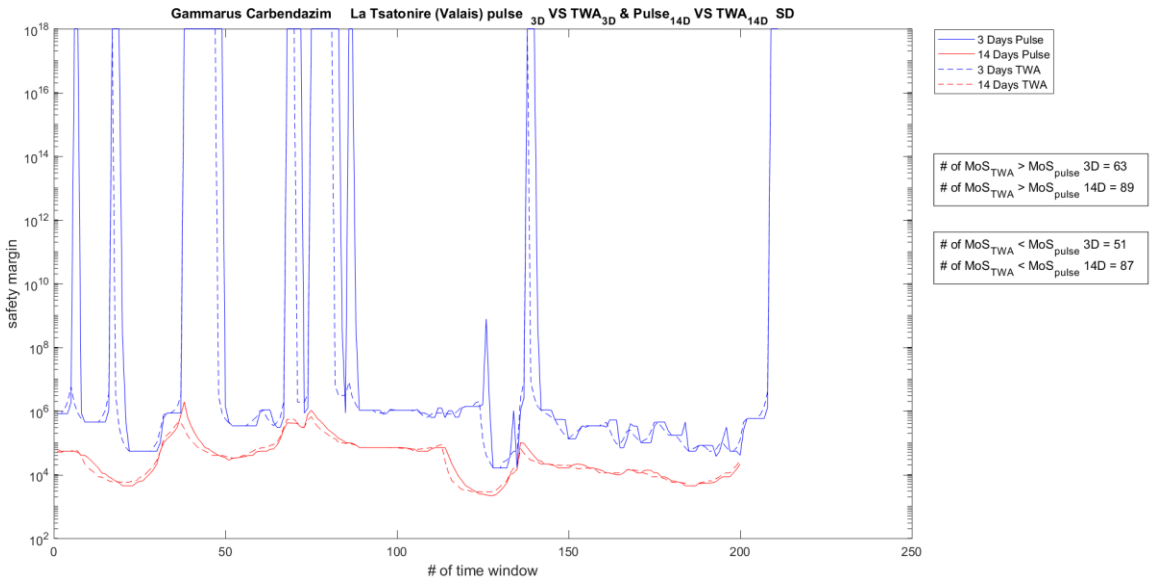


Figure 7. Margins of Safety with GUTS-SIC-SD pulse, 3 day TWA and 14 day TWA

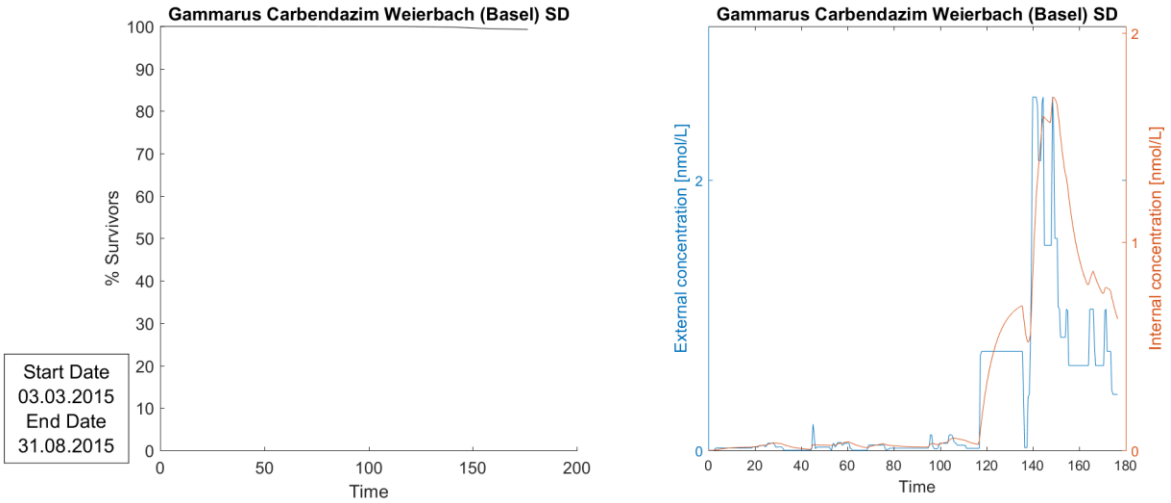


Figure 8. The simulated survival of *Gammarus pulex* for Carbendazim in Weierbach (Basel) with GUTS-SIC-SD for the full realistic exposure scenario (left) and the corresponding external and internal concentrations (right).

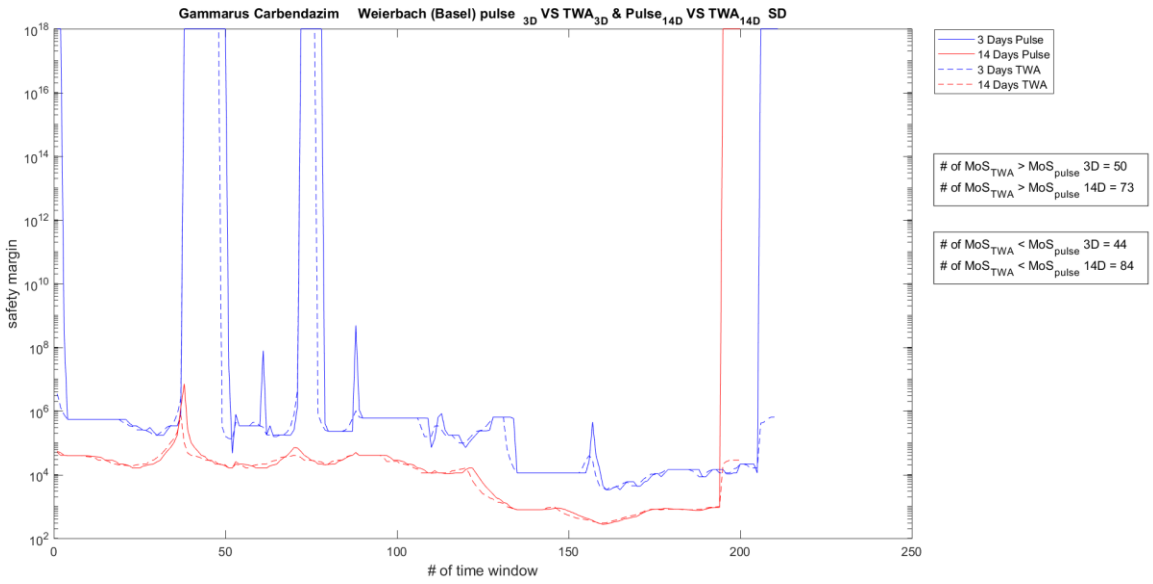


Figure 9. Margins of Safety with GUTS-SIC-SD pulse, 3 day TWA and 14 day TWA

Parameters and Confidence Intervals for *Gammarus pulex* and Chlorpyrifos

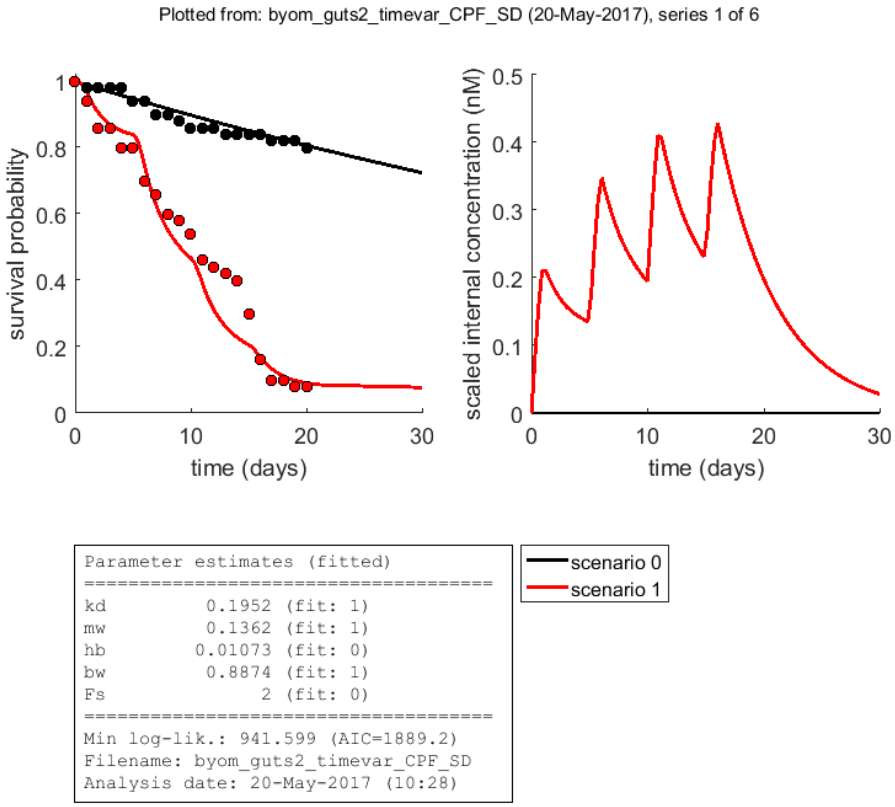


Figure 10. Graphs for *Gammarus pulex* and Chlorpyrifos estimated GUTS-SIC-SD parameters for exposure scenario 0 and 1

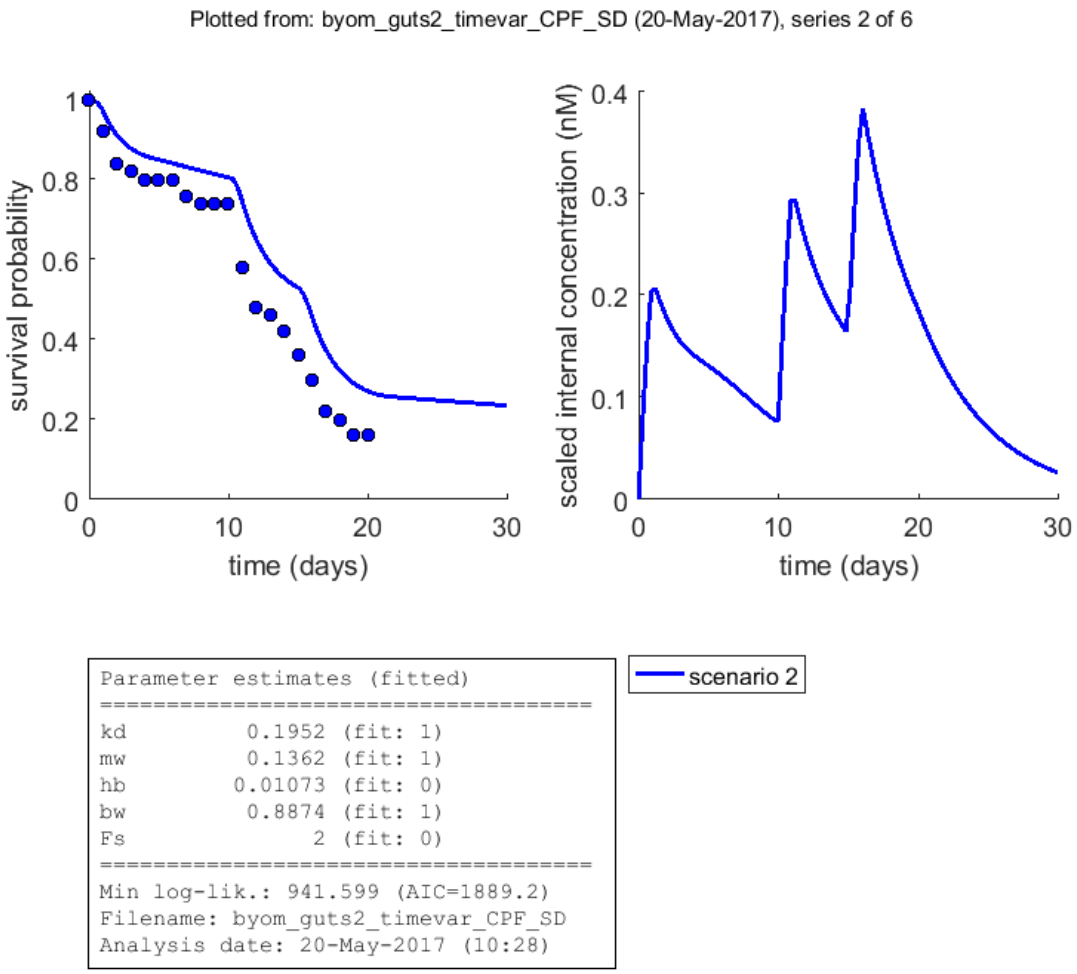


Figure 11. Graphs for *Gammarus pulex* and Chlorpyrifos estimated GUTS-SIC-SD parameters for exposure scenario 2

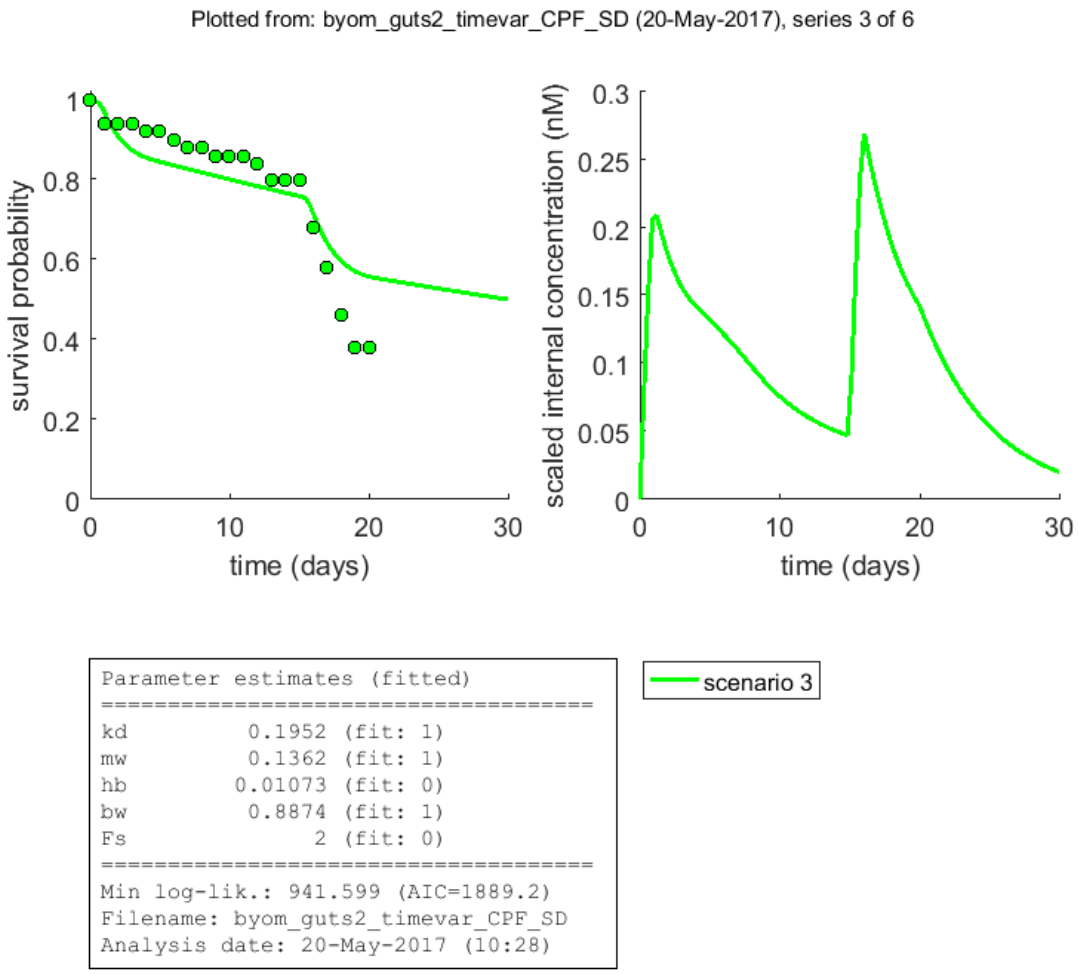


Figure 12. Graphs for *Gammarus pulex* and Chlorpyrifos estimated GUTS-SIC-SD parameters for exposure scenario 3

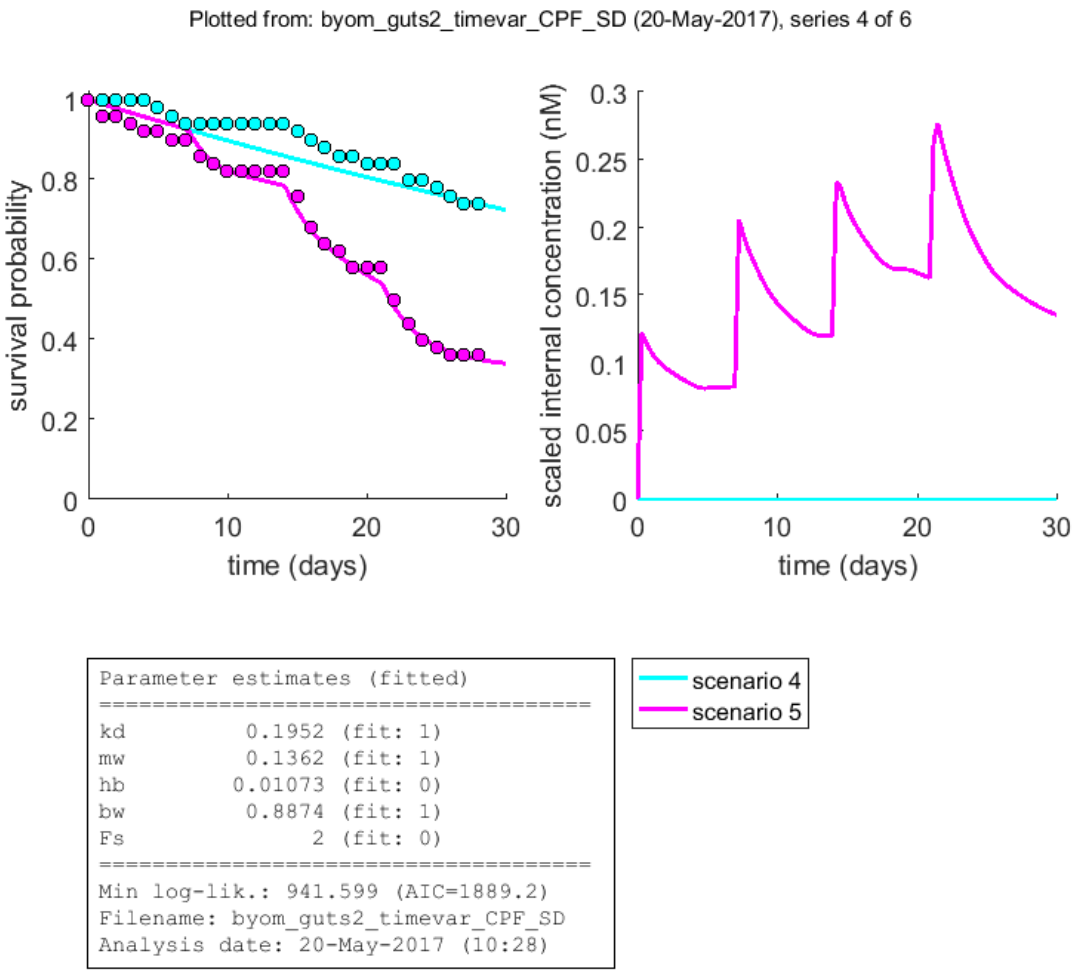


Figure 13. Graphs for *Gammarus pulex* and Chlorpyrifos estimated GUTS-SIC-SD parameters for exposure scenarios 4 and 5

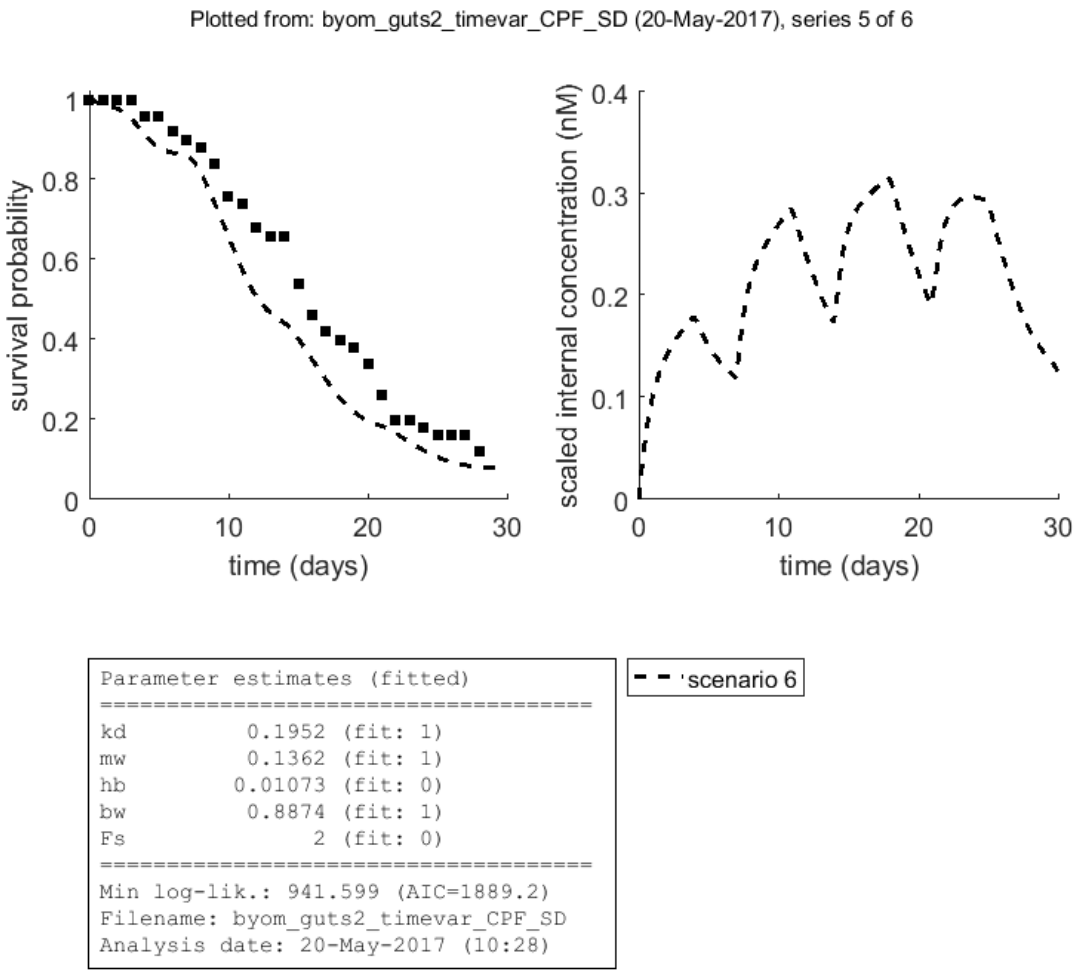


Figure 14. Graphs for *Gammarus pulex* and Chlorpyrifos estimated GUTS-SIC-SD parameters for exposure scenario 6

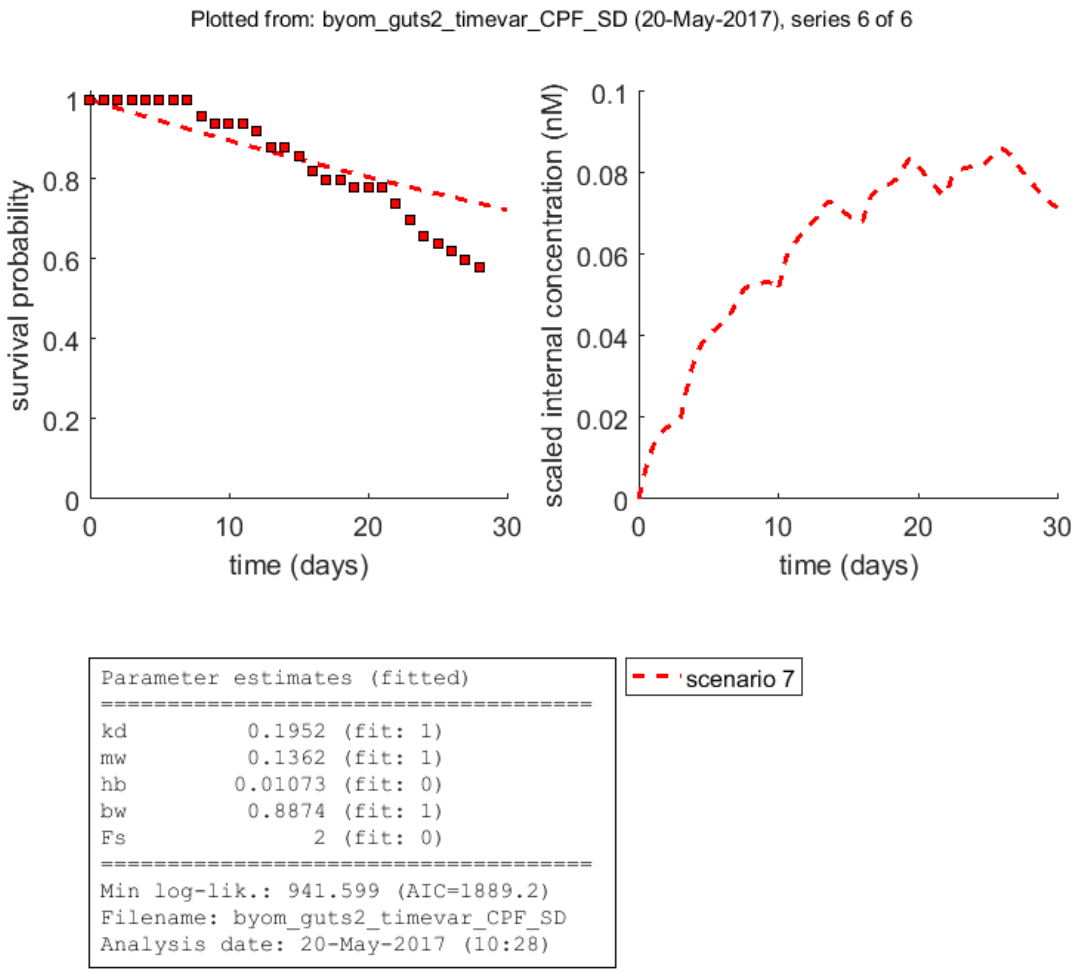


Figure 15. Graphs for *Gammarus pulex* and Chlorpyrifos estimated GUTS-SIC-SD parameters for exposure scenario 7

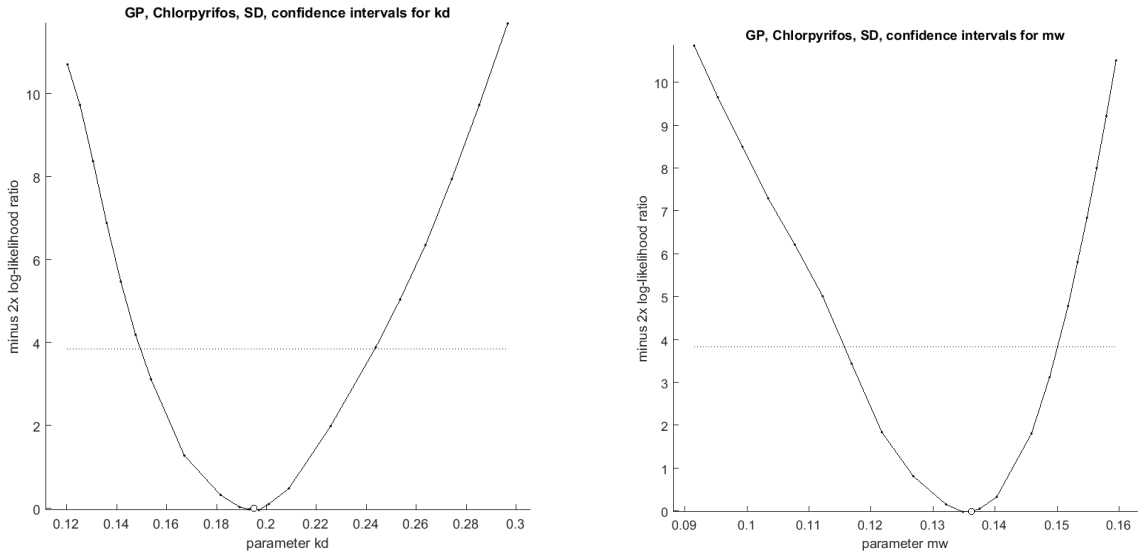


Figure 16. Confidence Intervals for *Gammarus pulex* and Chlorpyrifos for GUTS-SIC-SD parameter kd and mw

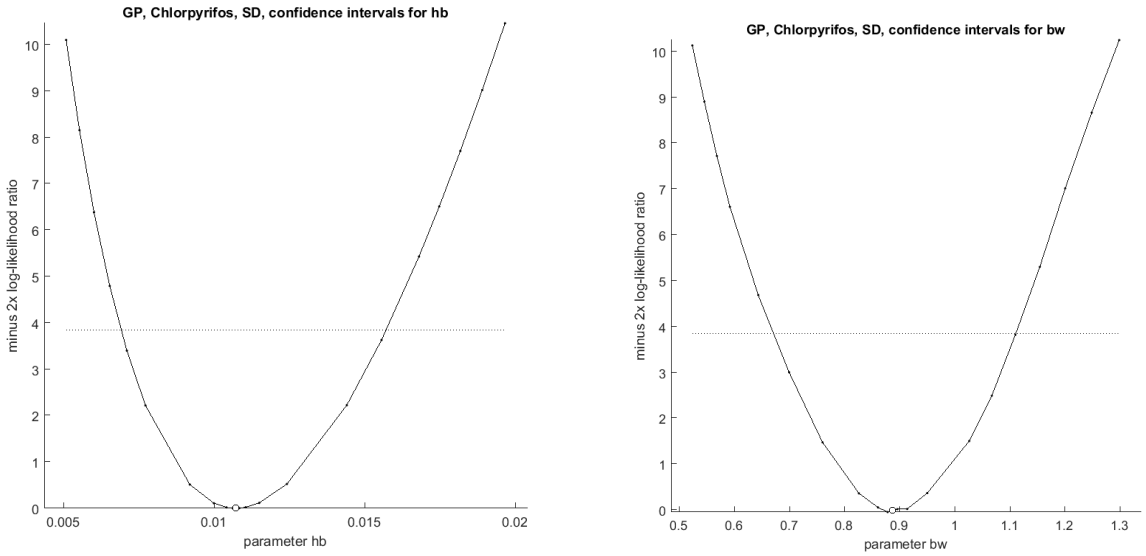


Figure 17. Confidence Intervals for *Gammarus pulex* and Chlorpyrifos for GUTS-SIC-SD parameter hb and bw

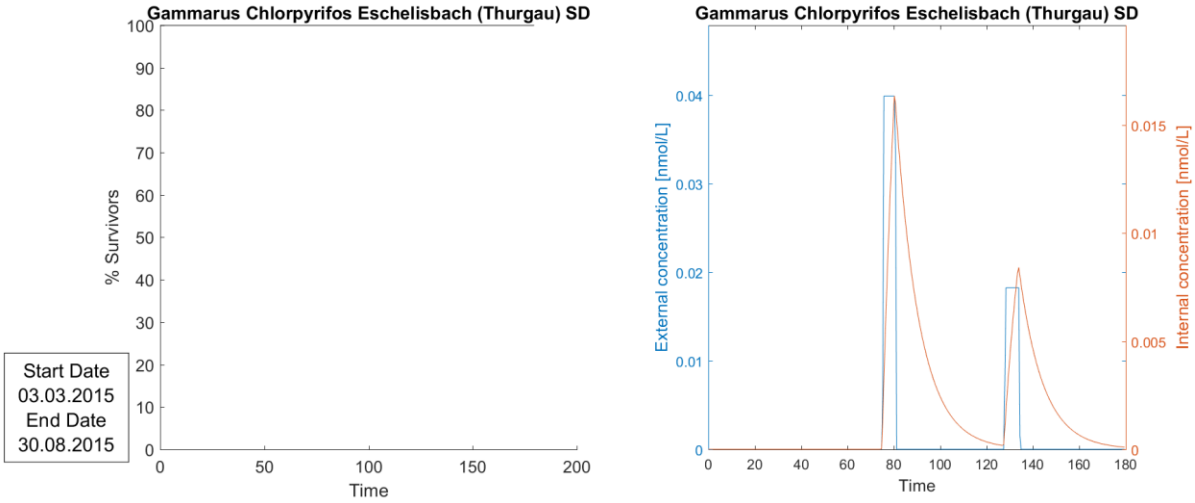


Figure 18. The simulated survival of *Gammarus pulex* for Chloprpyrifos in Eschelisbach (Thurgau) with GUTS-SIC-SD for the full realistic exposure scenario (left) and the corresponding external and internal concentrations (right).

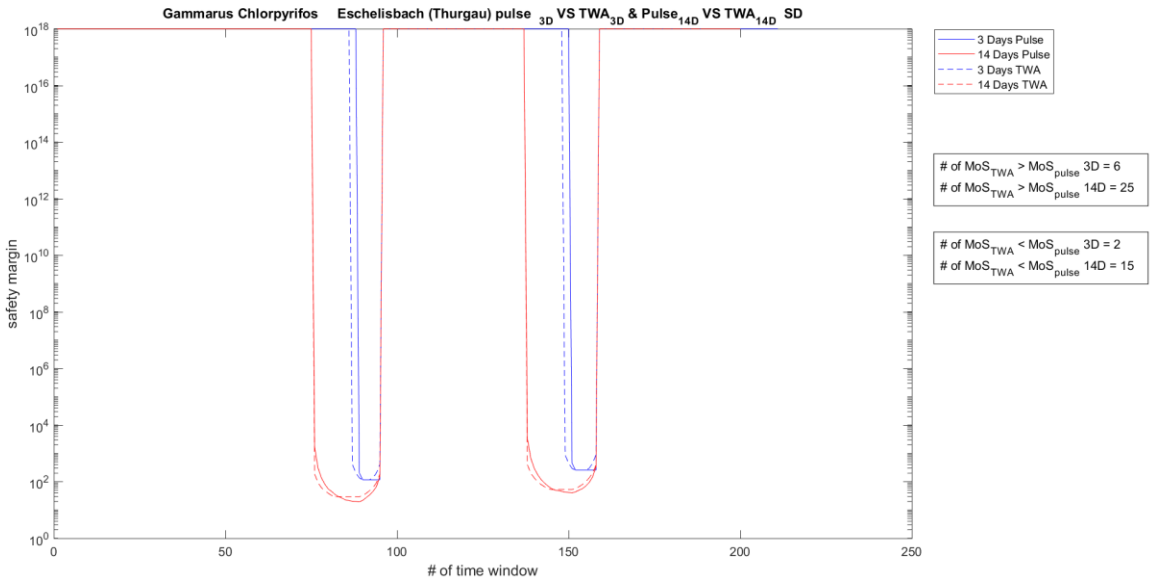


Figure 19. Margins of Safety with GUTS-SIC-SD pulse, 3 day TWA and 14 day TWA

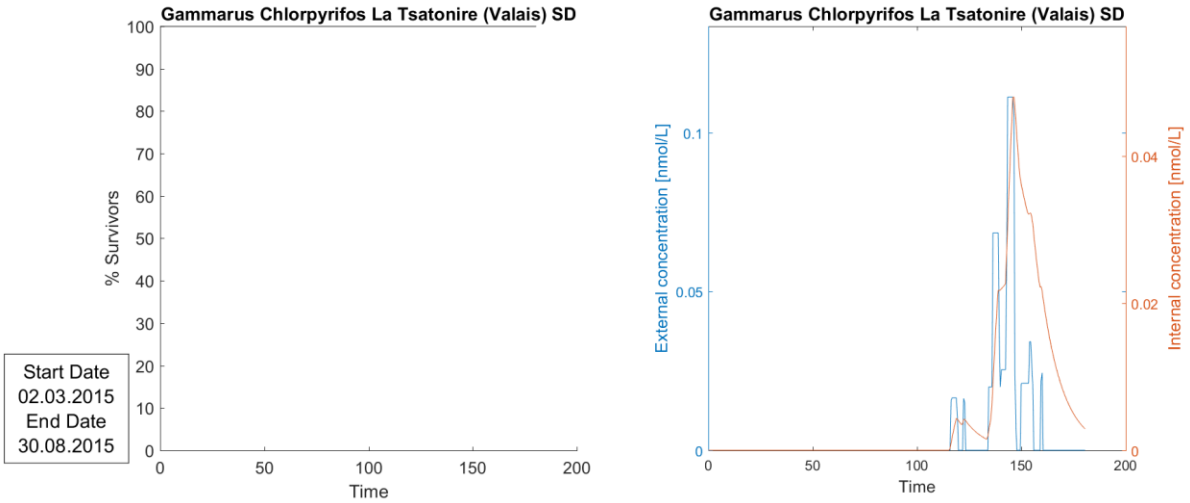


Figure 20. The simulated survival of *Gammarus pulex* for Chloprpyrifos in La Tsatonire (Valais) with GUTS-SIC-SD for the full realistic exposure scenario (left) and the corresponding external and internal concentrations (right).

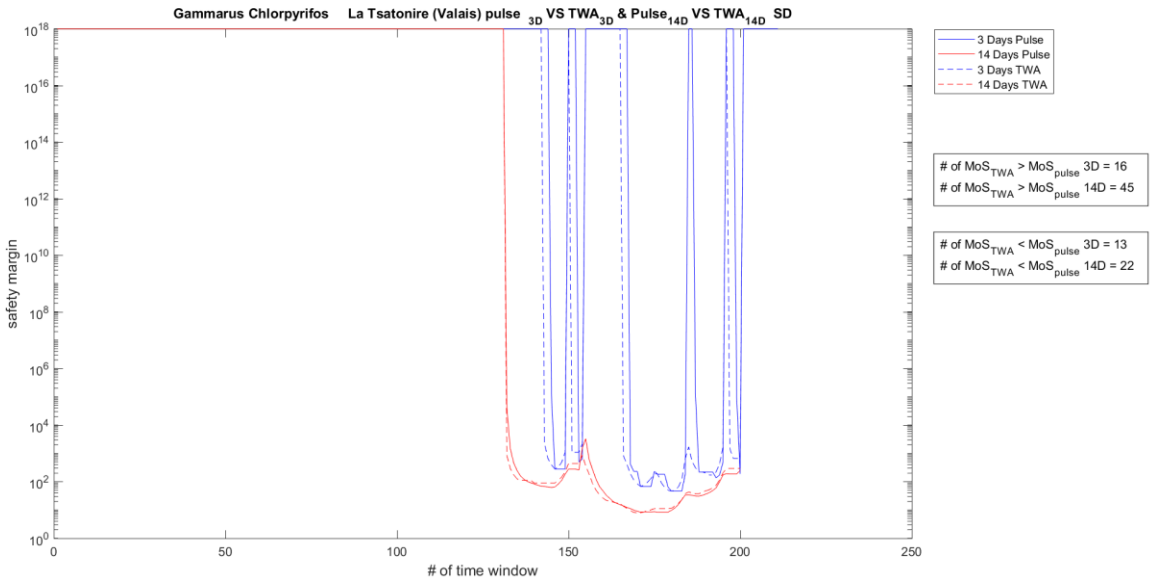


Figure 21. Margins of Safety with GUTS-SIC-SD pulse, 3 day TWA and 14 day TWA

Parameters and Confidence Intervals for *Gammarus pulex* and Diazinon

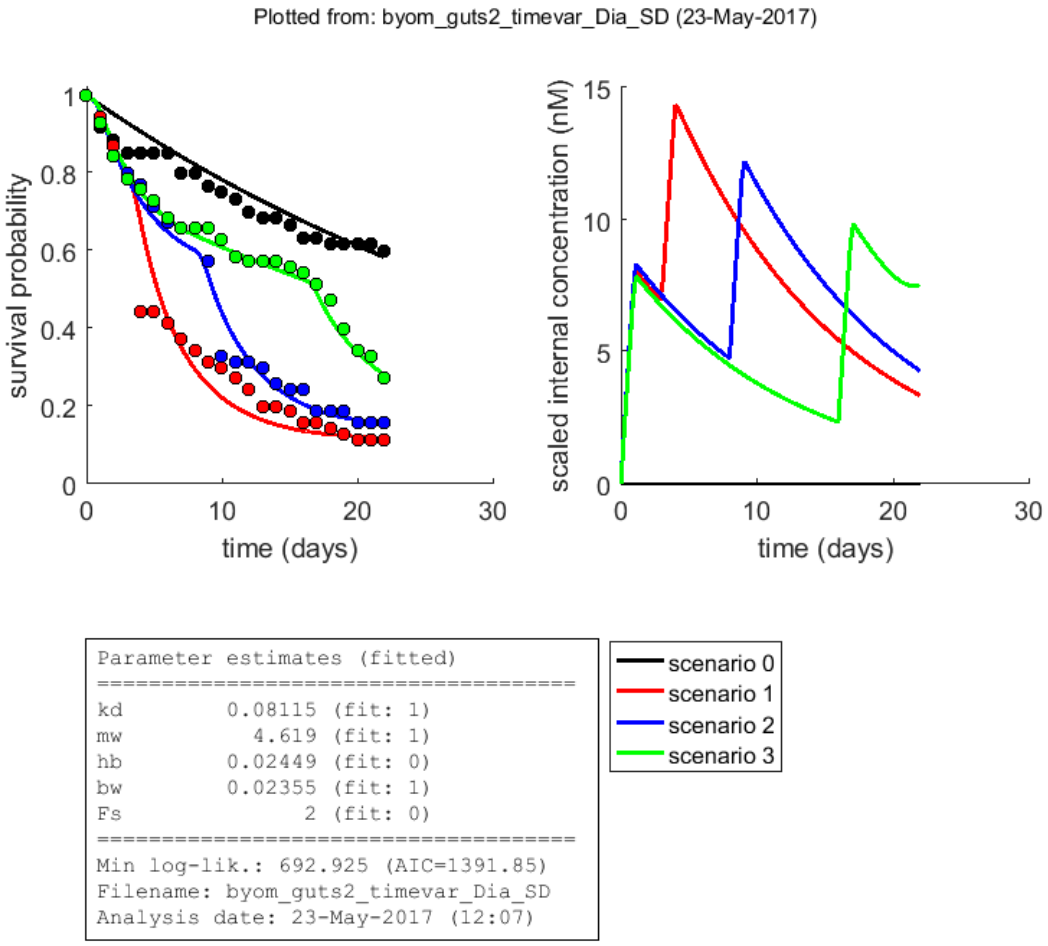


Figure 22. Graph for *Gammarus pulex* and Diazinon estimated GUTS-SIC-SD parameters

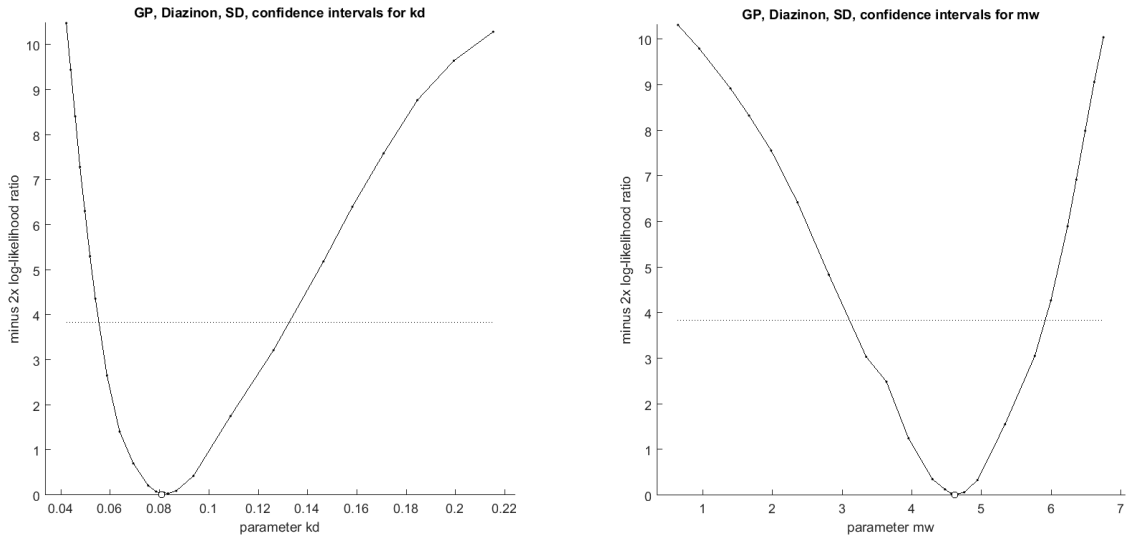


Figure 23. Confidence Intervals for *Gammarus pulex* and Diazinon for GUTS-SIC-SD parameter kd and mw

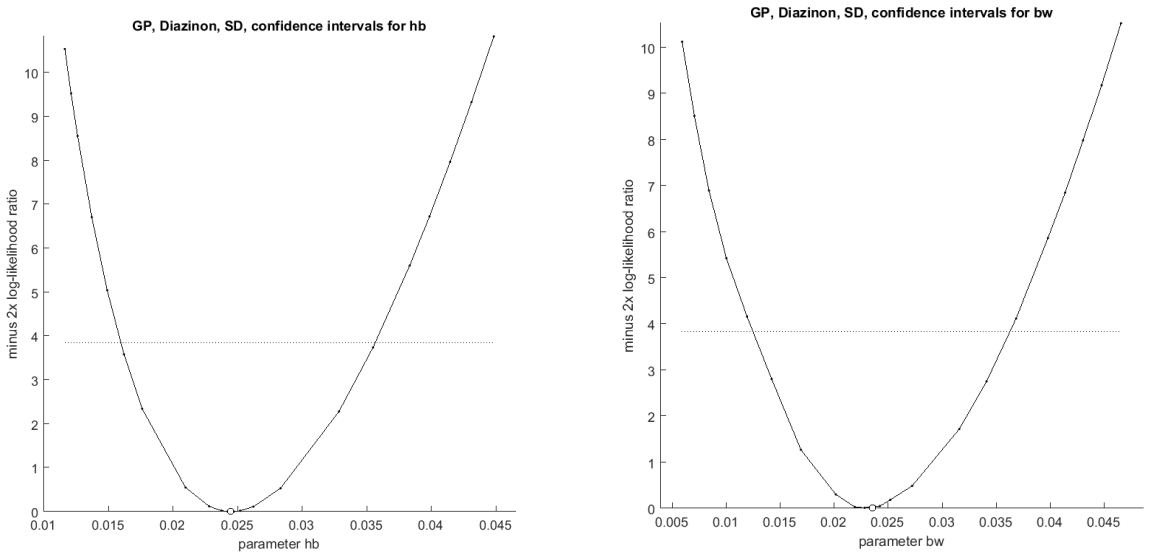


Figure 24. Confidence Intervals for *Gammarus pulex* and Diazinon for GUTS-SIC-SD parameter hb and bw

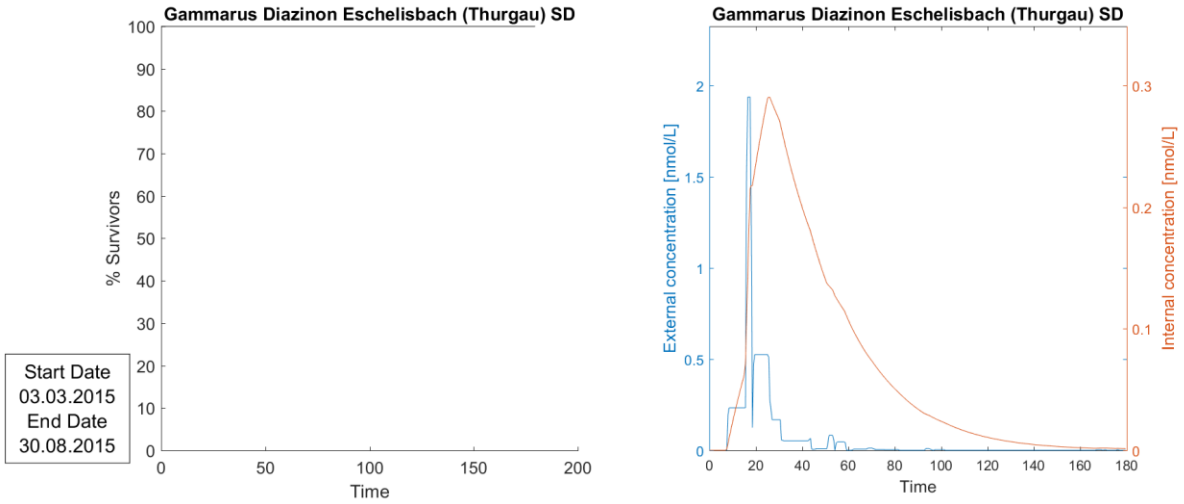


Figure 25. The simulated survival of *Gammarus pulex* for Diazinon in Eschelisbach (Thurgau) with GUTS-SIC-SD for the full realistic exposure scenario (left) and the corresponding external and internal concentrations (right).

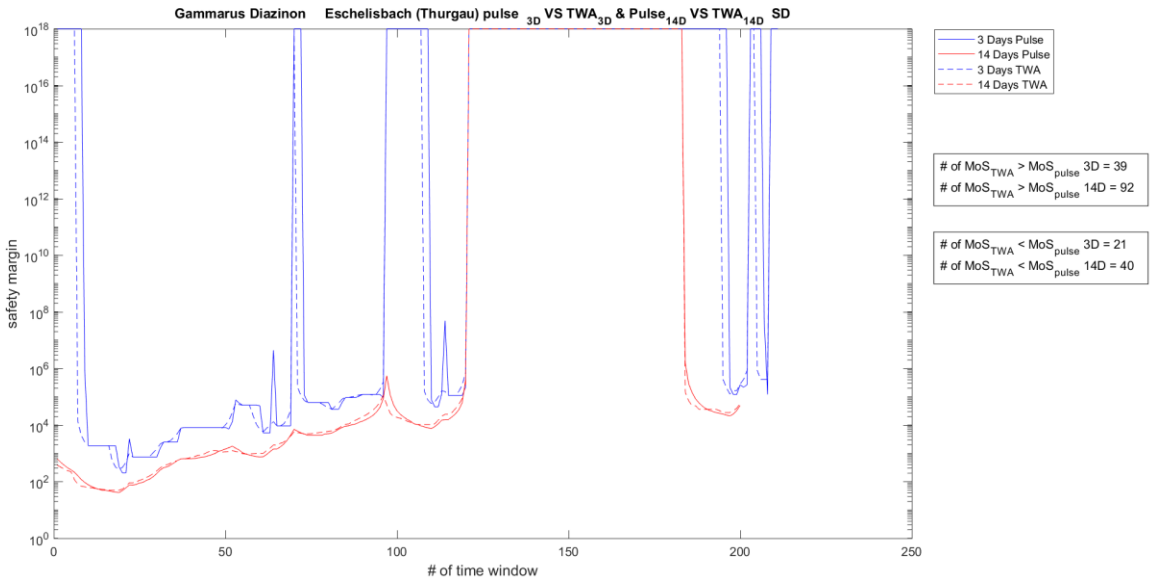


Figure 26. Margins of Safety with GUTS-SIC-SD pulse, 3 day TWA and 14 day TWA

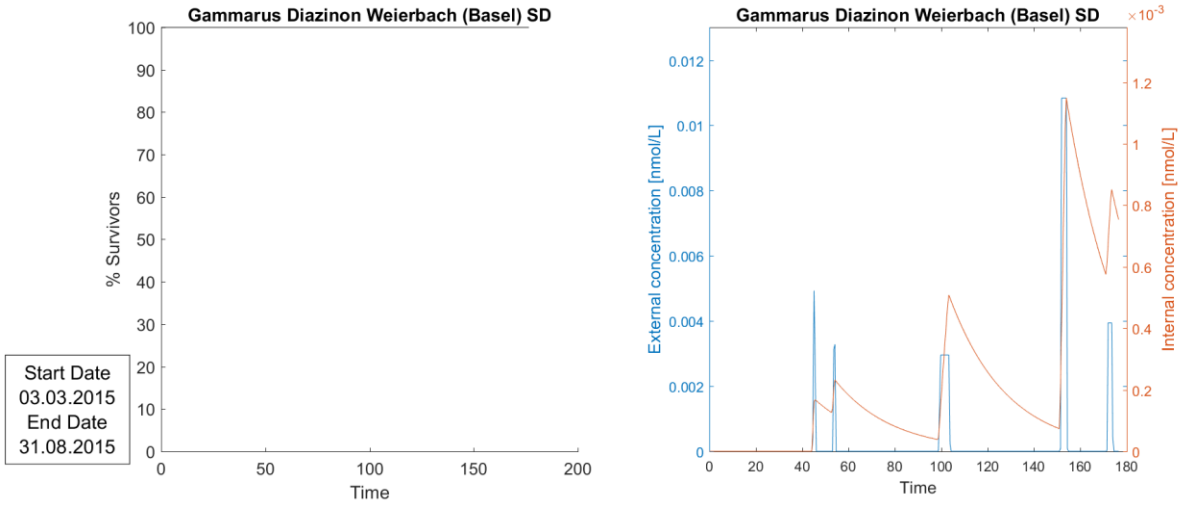


Figure 27. The simulated survival of *Gammarus pulex* for Diazinon in Weierbach (Basel) with GUTS-SIC-SD for the full realistic exposure scenario (left) and the corresponding external and internal concentrations (right).

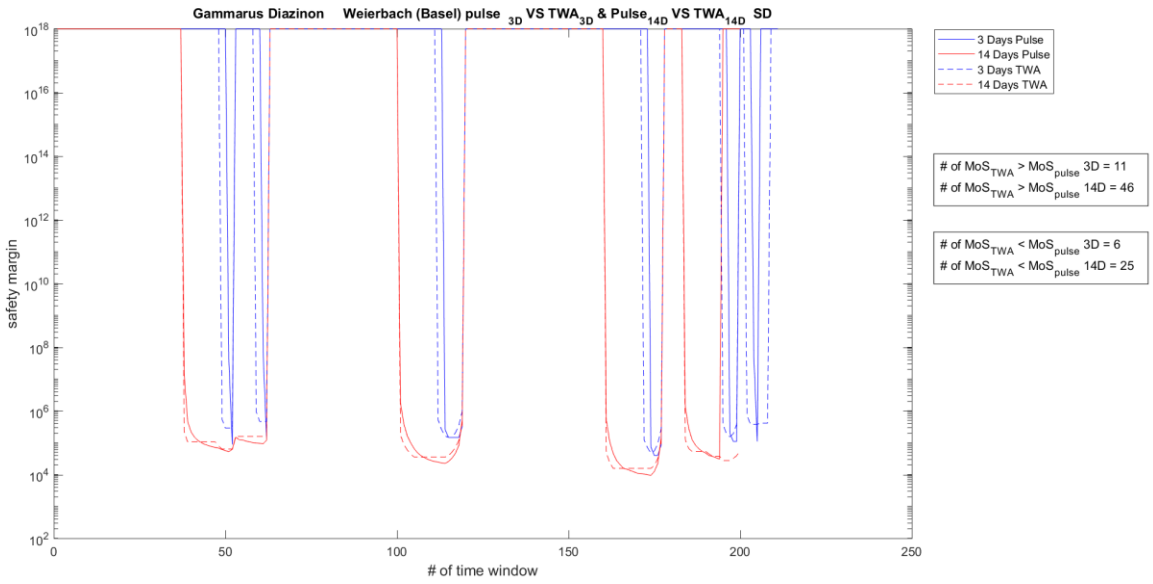


Figure 28. Margins of Safety with GUTS-SIC-SD pulse, 3 day TWA and 14 day TWA

Parameters and Confidence Intervals for *Gammarus pulex* and Dimethoate

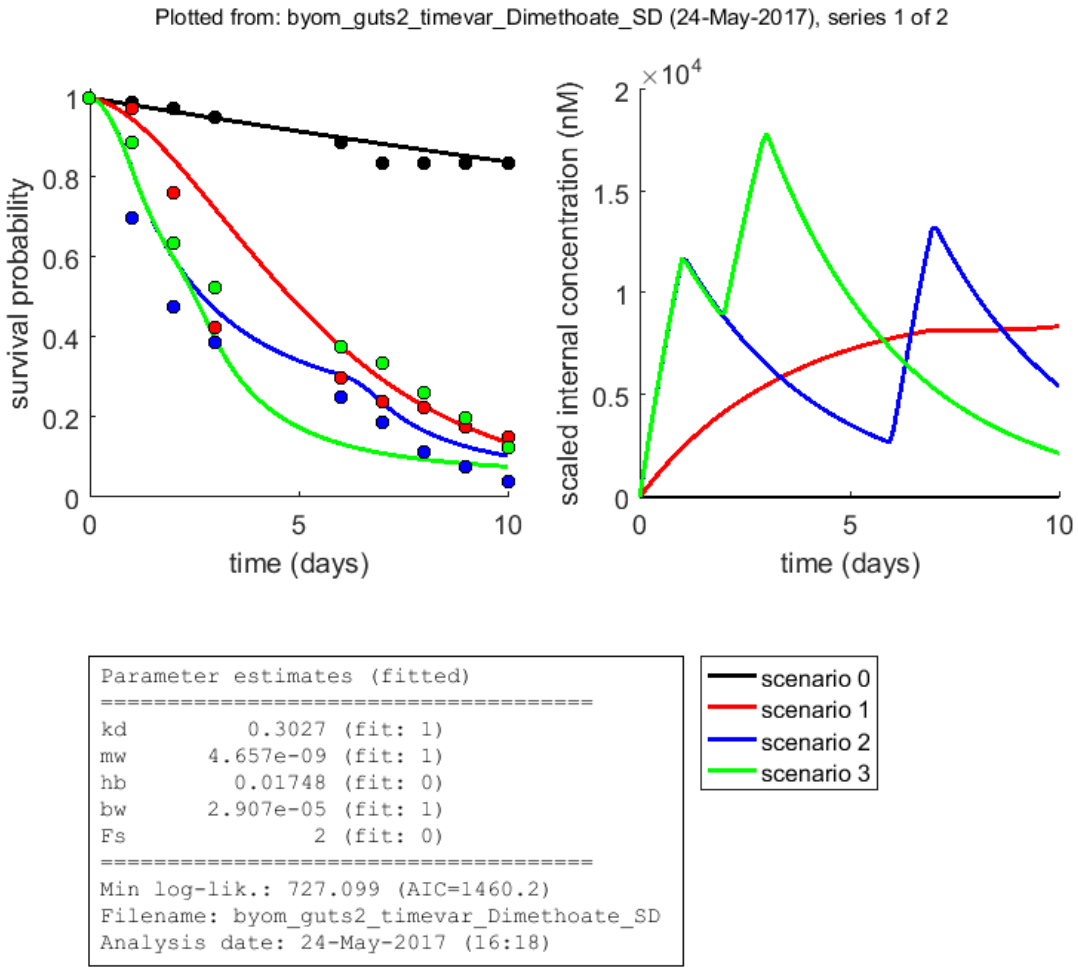


Figure 29. Graph for *Gammarus pulex* and Dimethoate estimated GUTS-SIC-SD parameters for scenarios 0 to 3

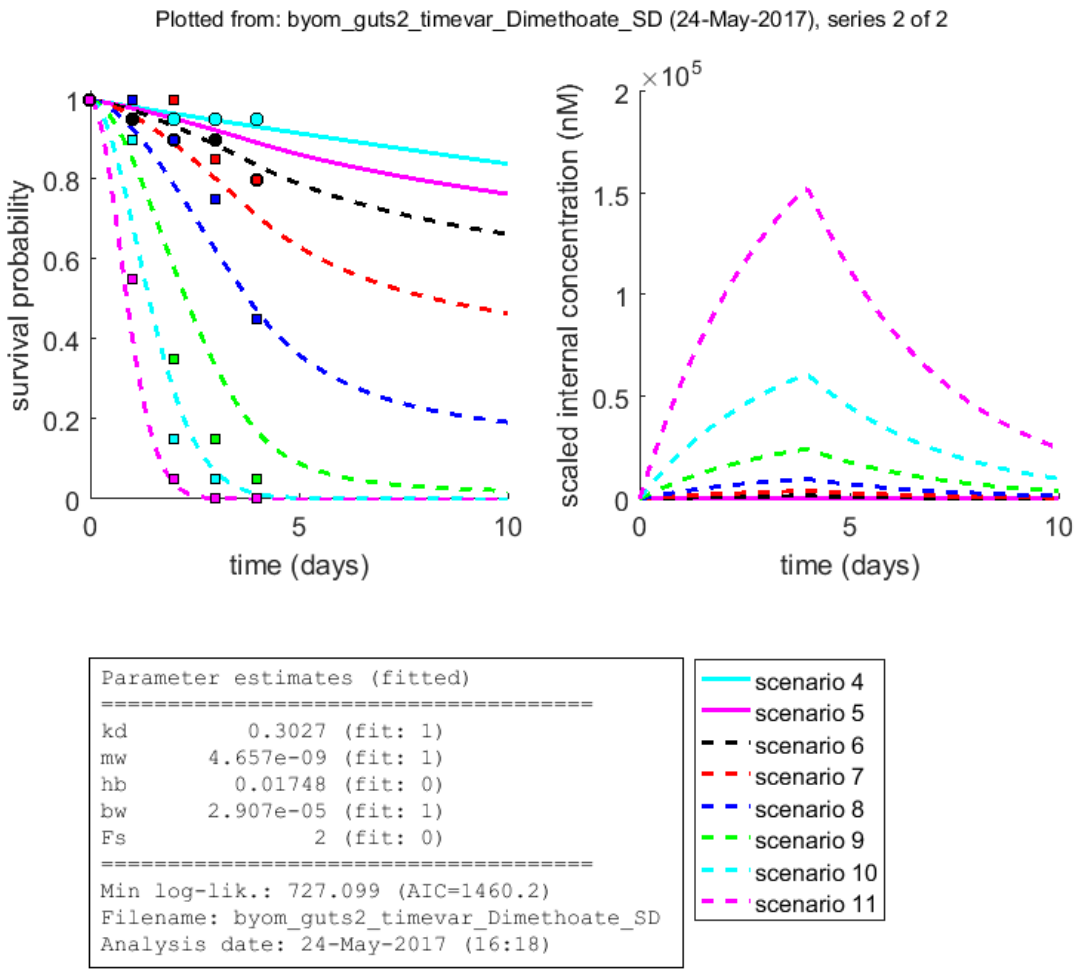


Figure 30. Graph for *Gammarus pulex* and Dimethoate estimated GUTS-SIC-SD parameters for scenarios 4 to 11

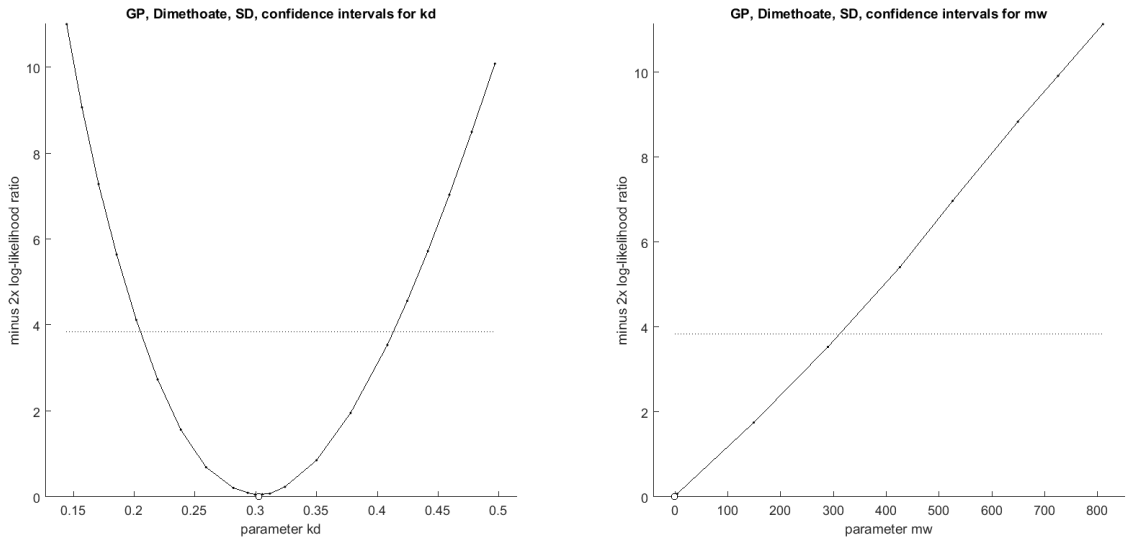


Figure 31. Confidence Intervals for *Gammarus pulex* and Dimethoate for GUTS-SIC-SD parameter kd and mw

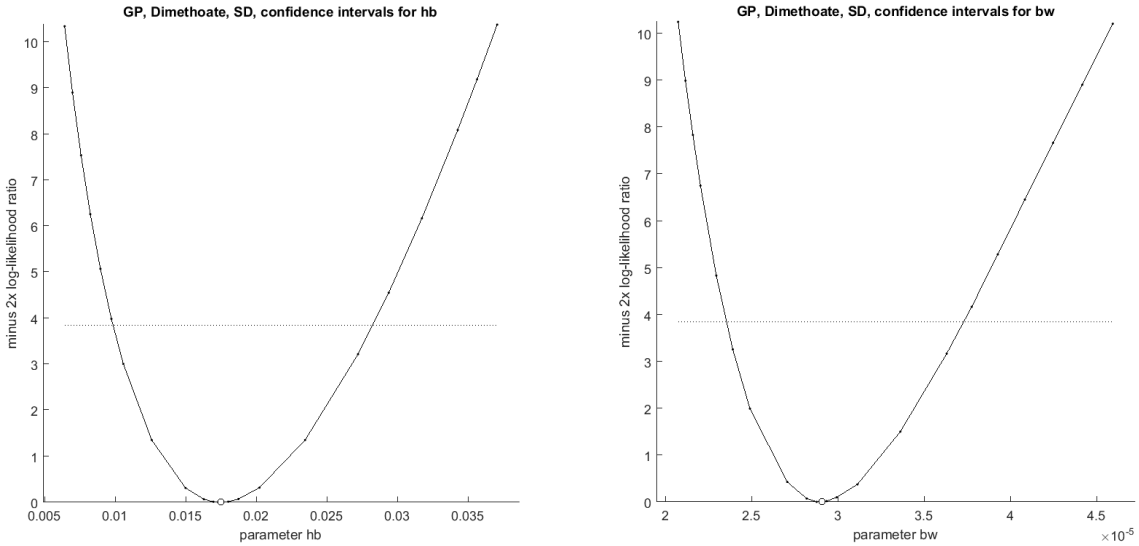


Figure 32. Confidence Intervals for *Gammarus pulex* and Dimethoate for GUTS-SIC-SD parameter hb and bw

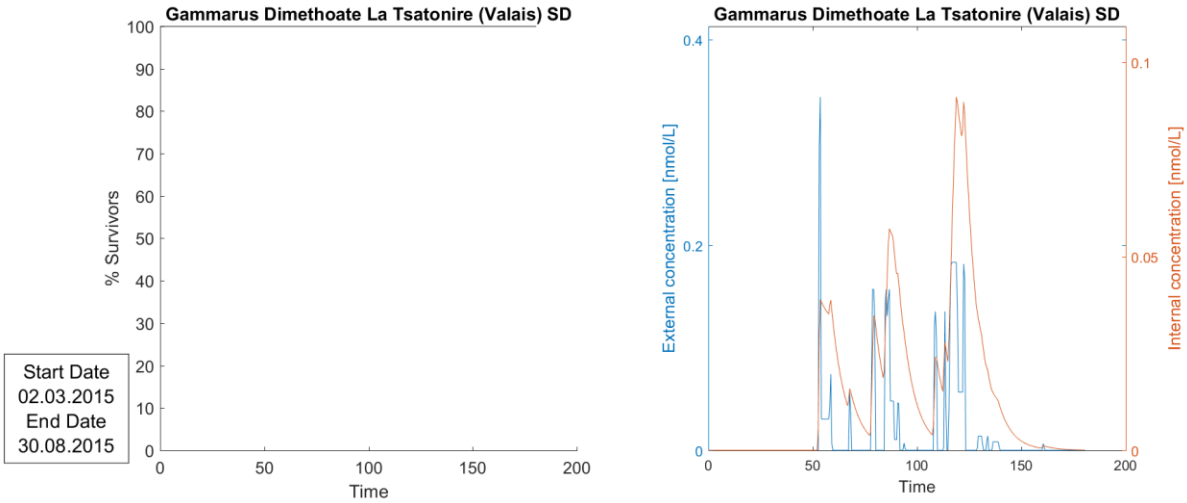


Figure 33. The simulated survival of *Gammarus pulex* for Dimethoate in La Tsatonire (Valais) with GUTS-SIC-SD for the full realistic exposure scenario (left) and the corresponding external and internal concentrations (right).

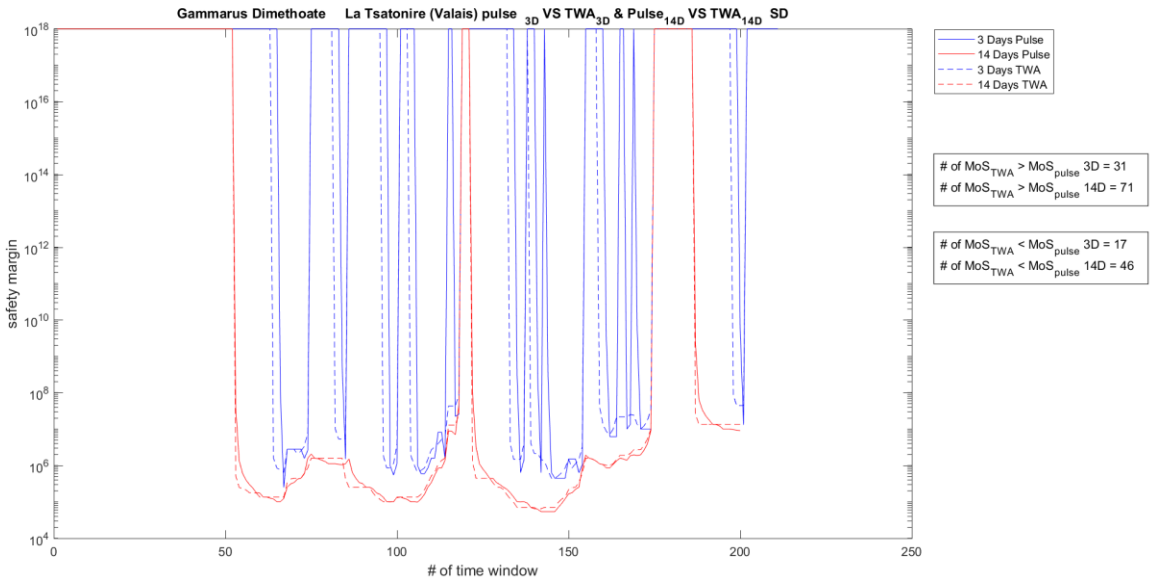


Figure 34. Margins of Safety with GUTS-SIC-SD pulse, 3 day TWA and 14 day TWA

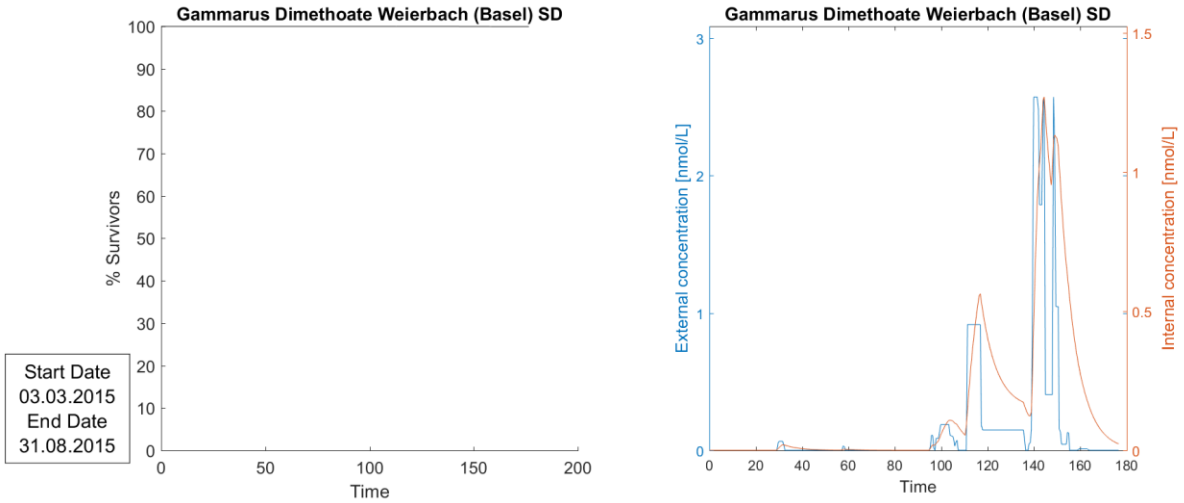


Figure 35. The simulated survival of *Gammarus pulex* for Dimethoate in Weierbach (Basel) with GUTS-SIC-SD for the full realistic exposure scenario (left) and the corresponding external and internal concentrations (right).

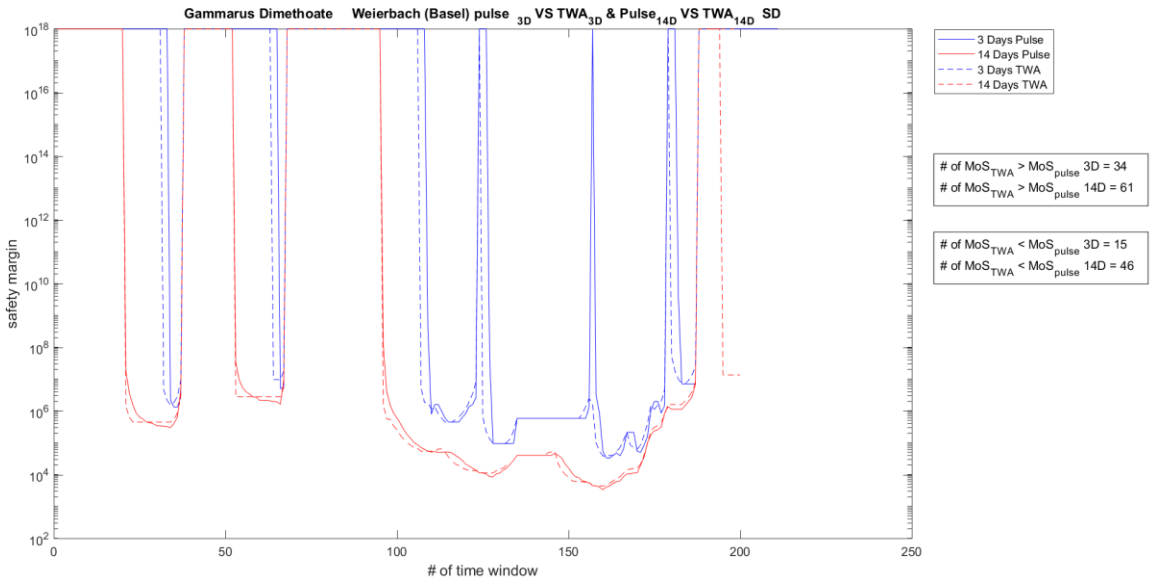


Figure 36. Margins of Safety with GUTS-SIC-SD pulse, 3 day TWA and 14 day TWA

Parameters and Confidence Intervals for *Gammarus pulex* and Imidacloprid

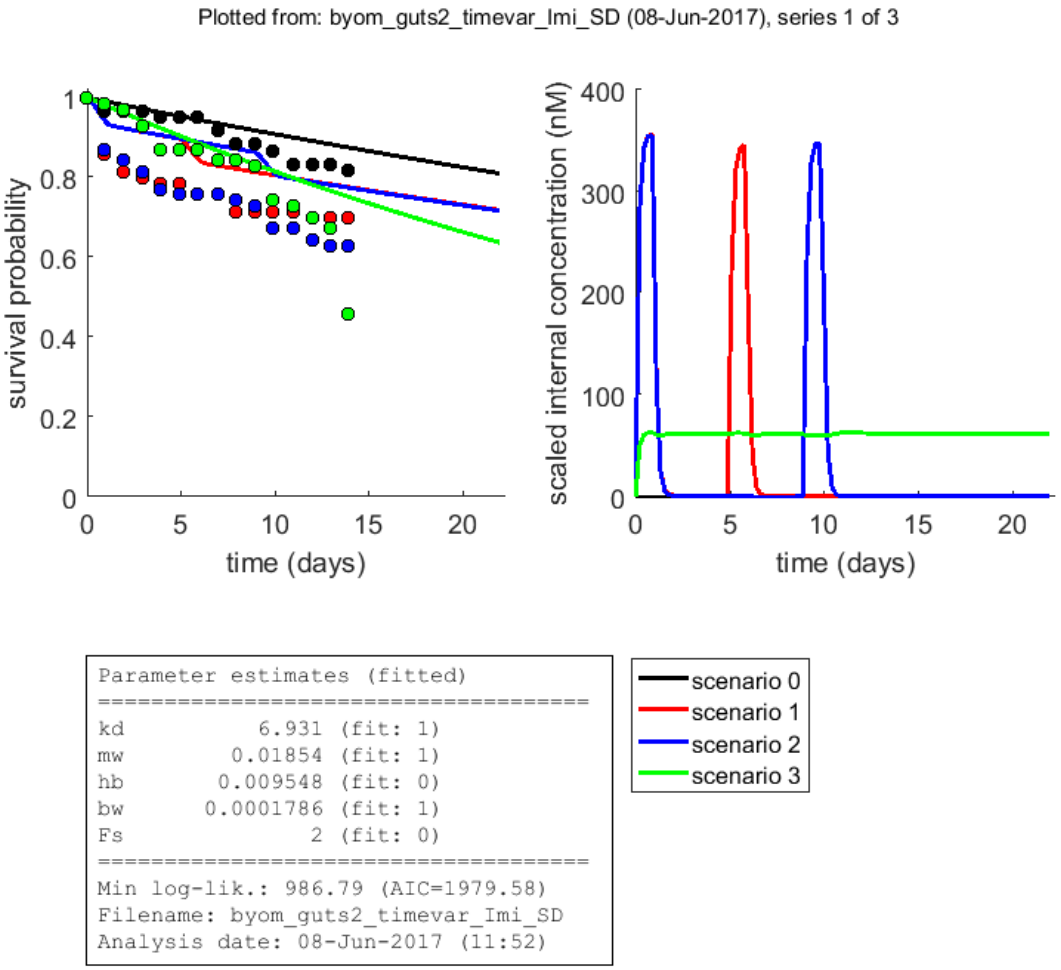
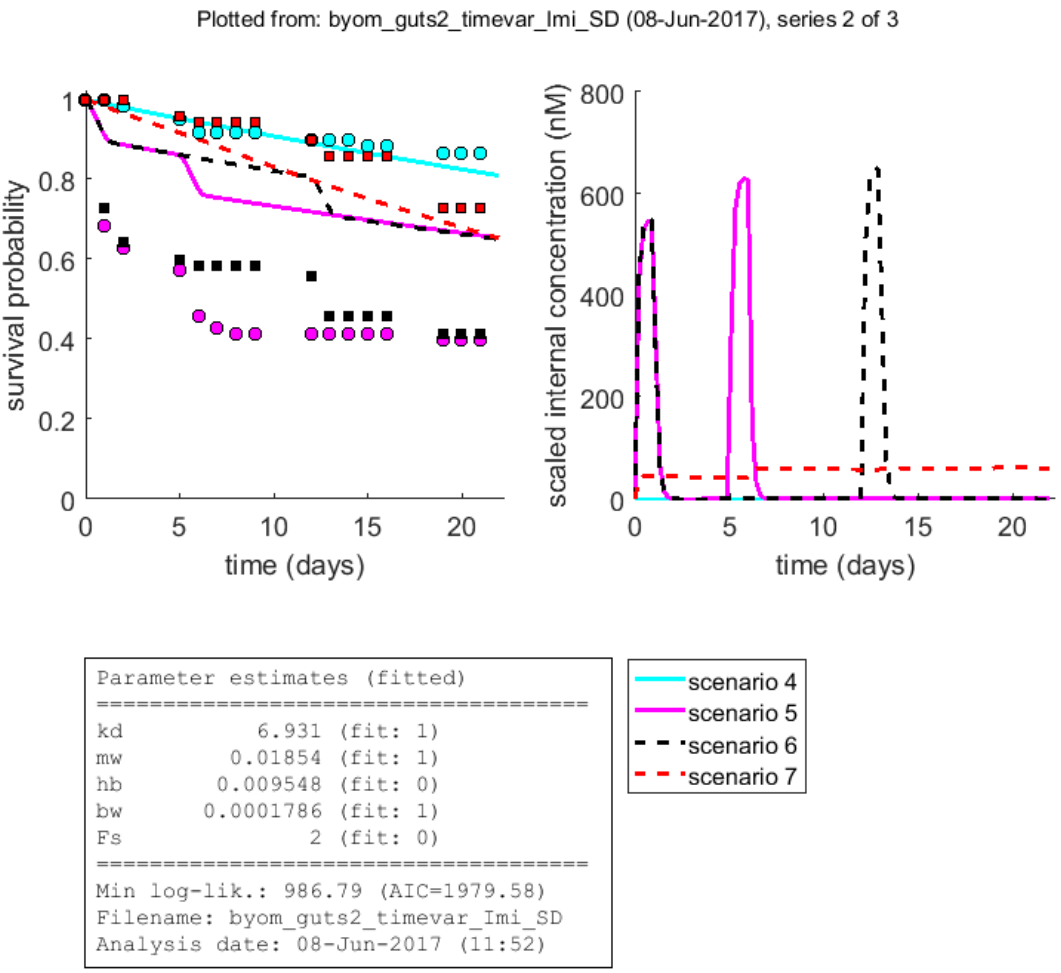


Figure 37. Graph for *Gammarus pulex* and Imidacloprid estimated GUTS-SIC-SD parameters for scenarios 0 to 3



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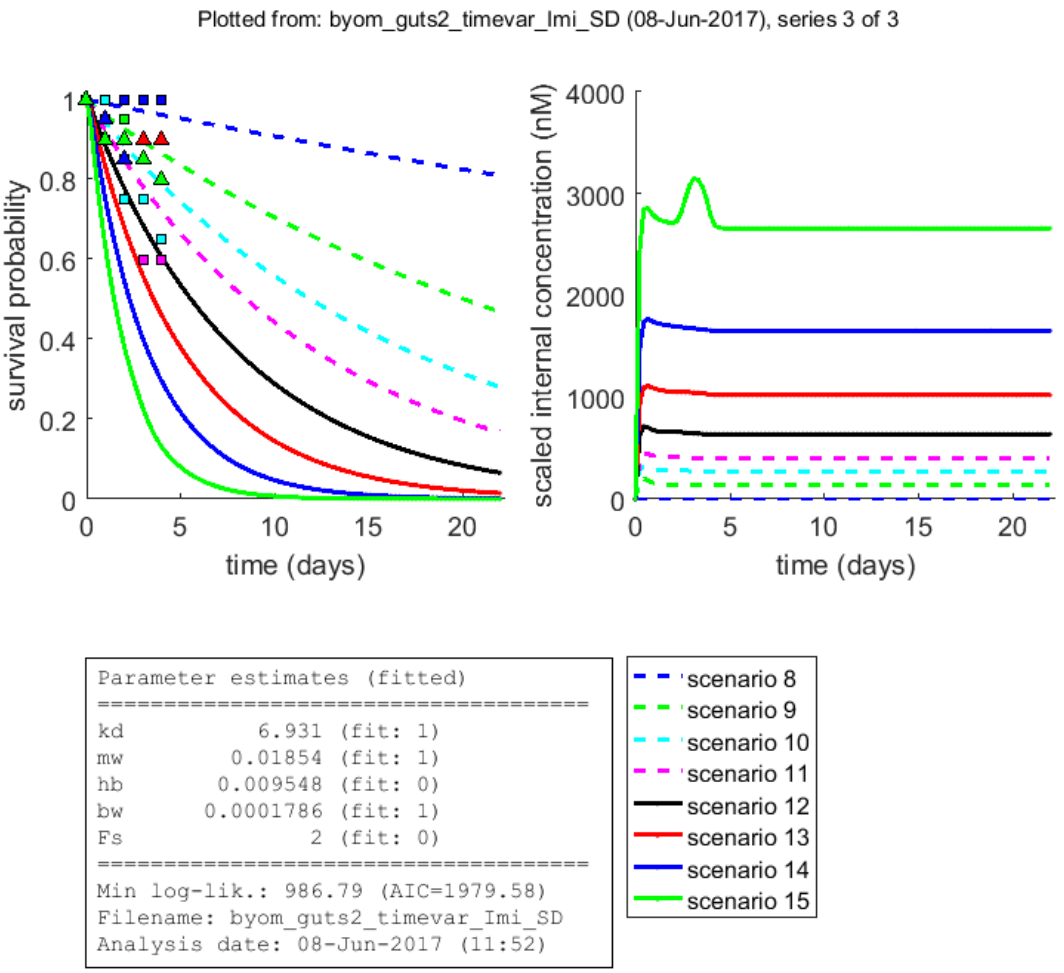

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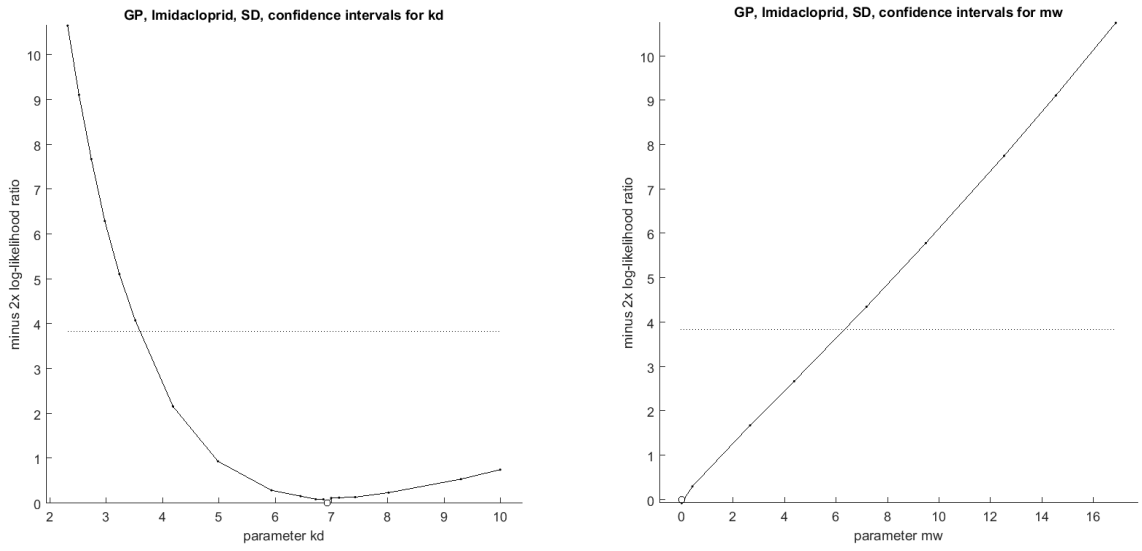

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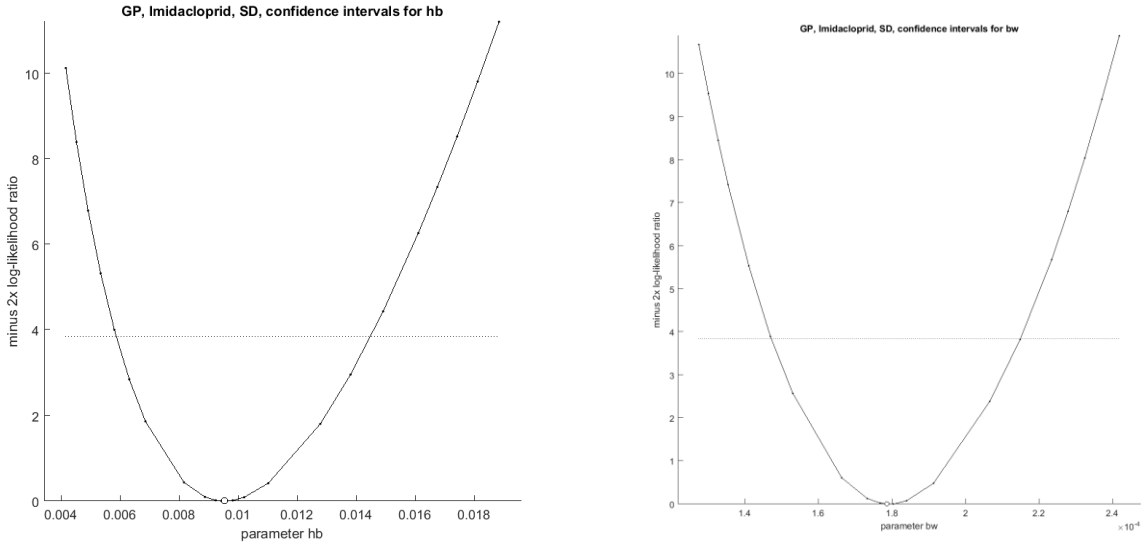

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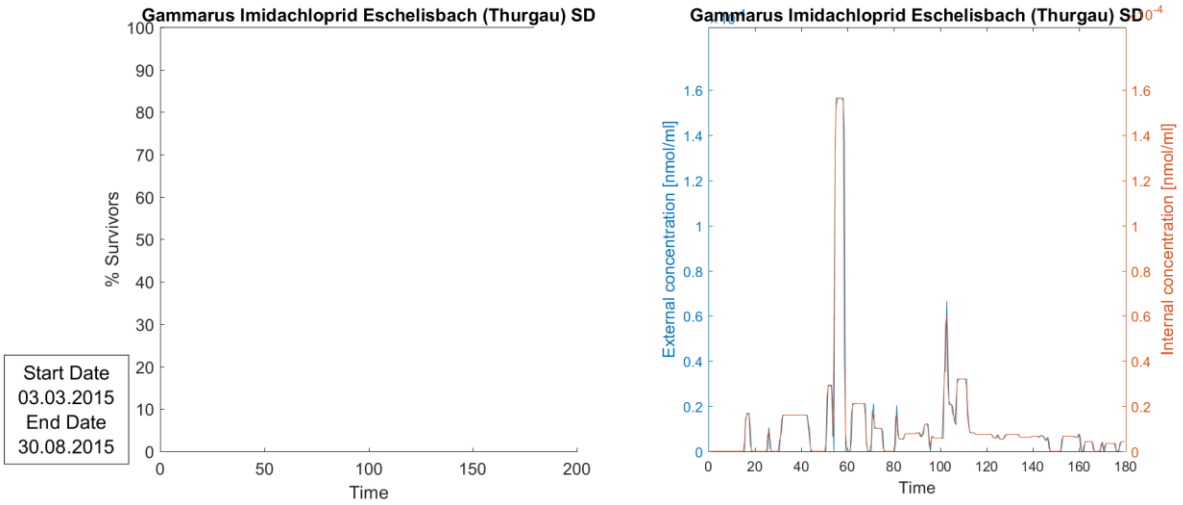

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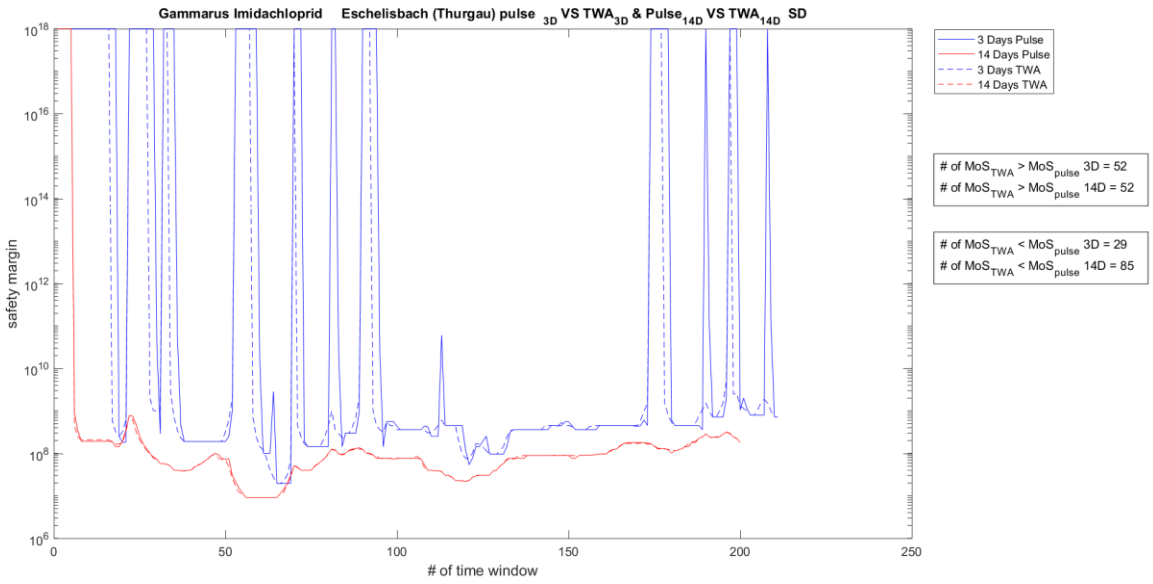

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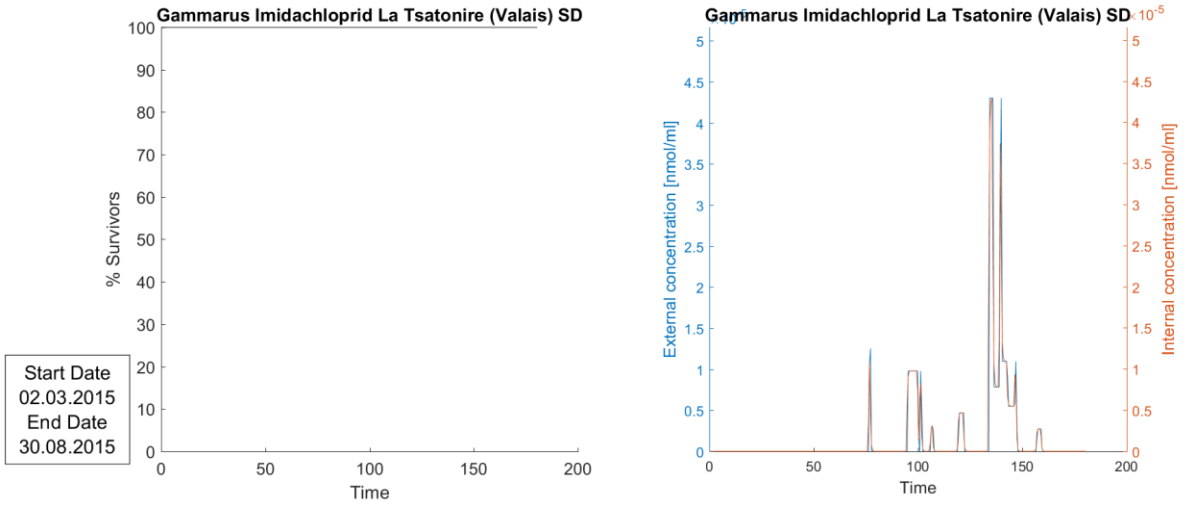

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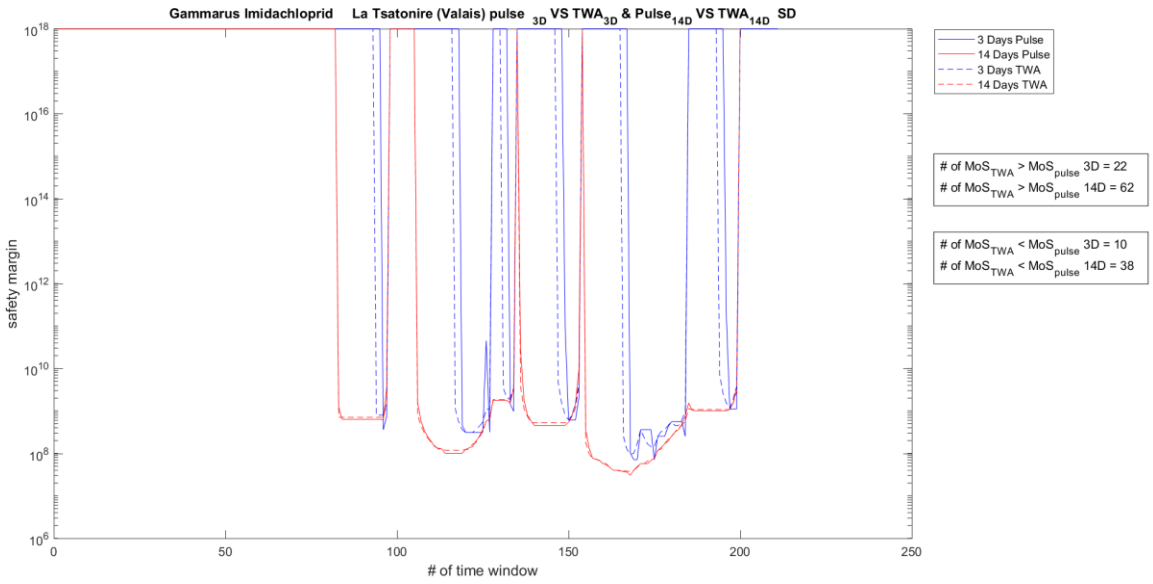

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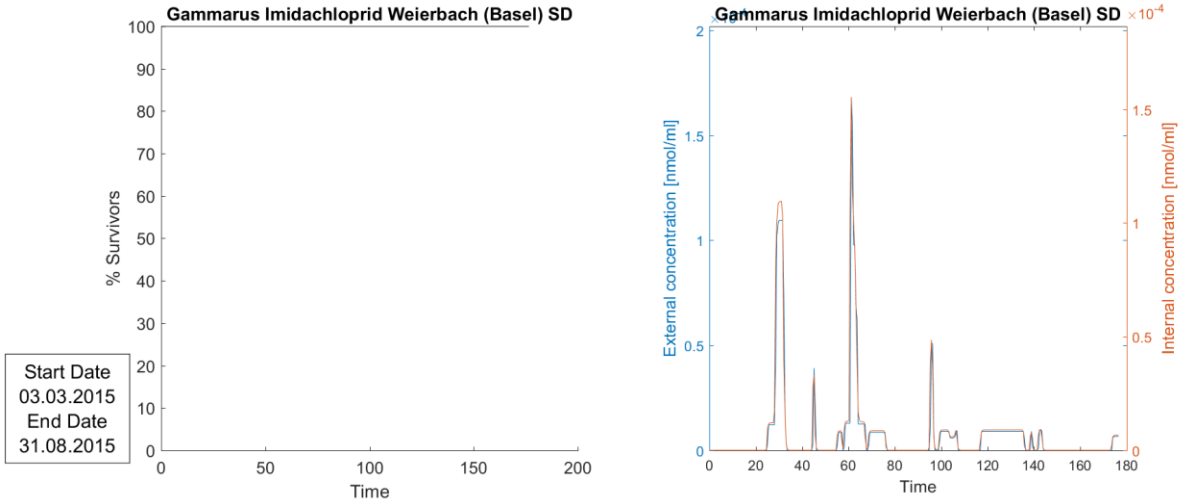

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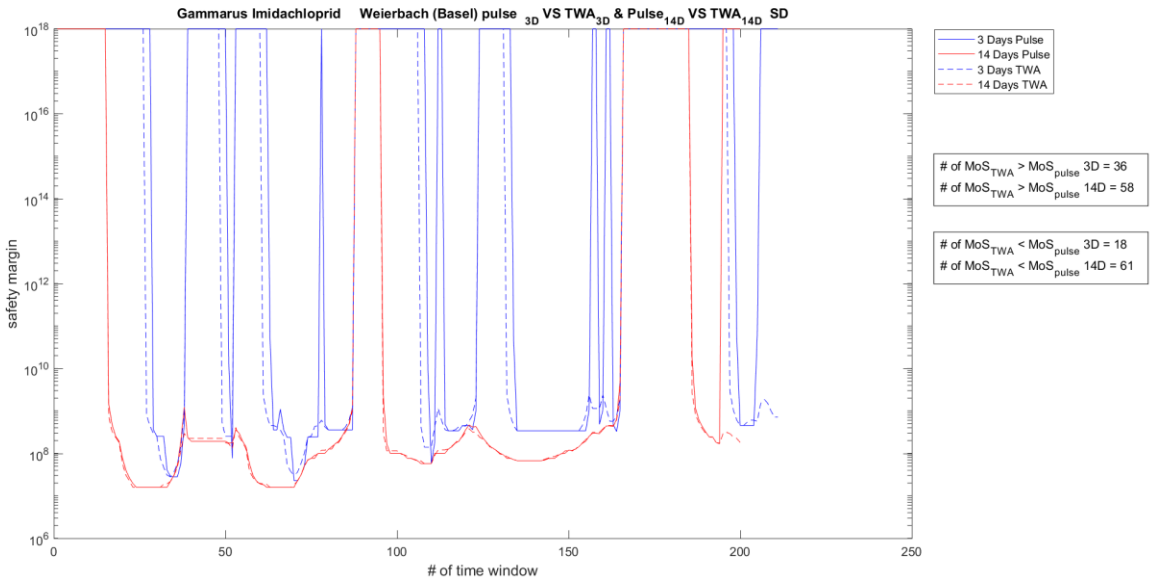

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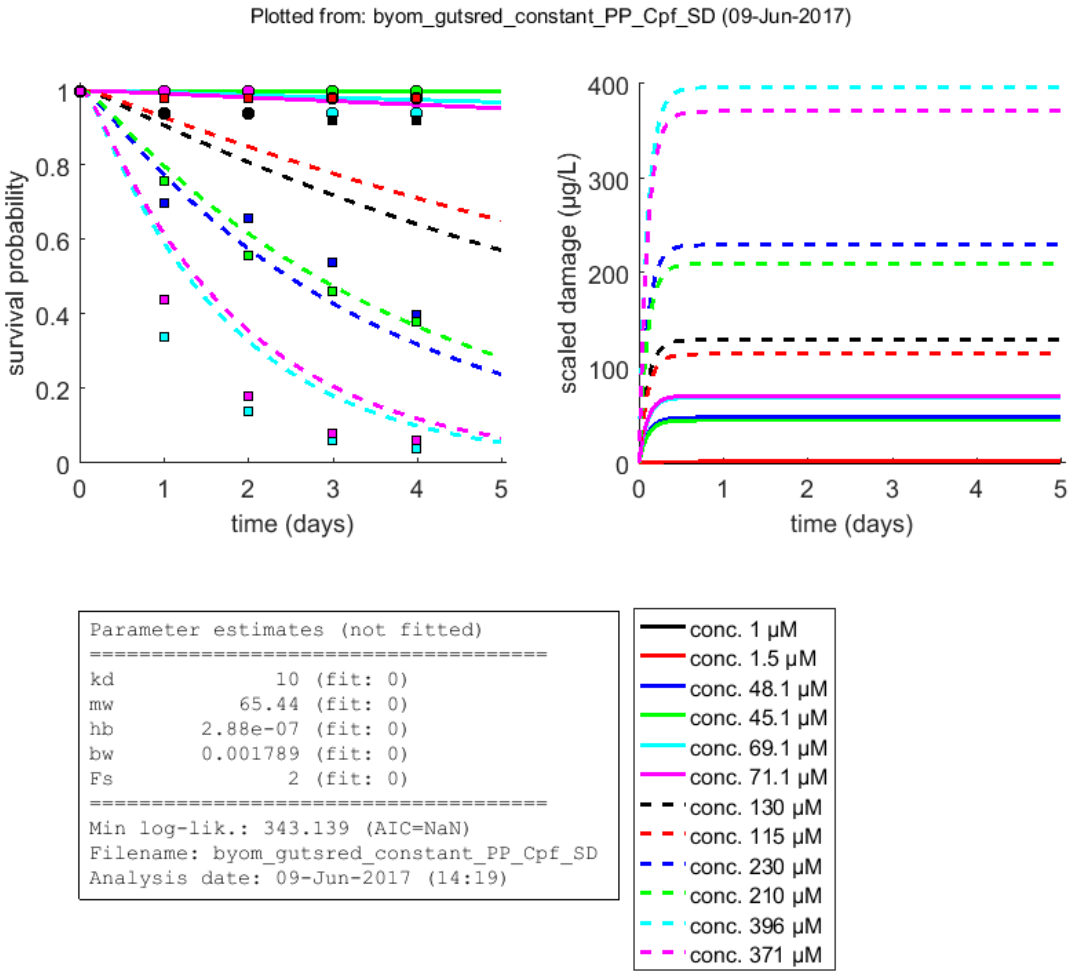

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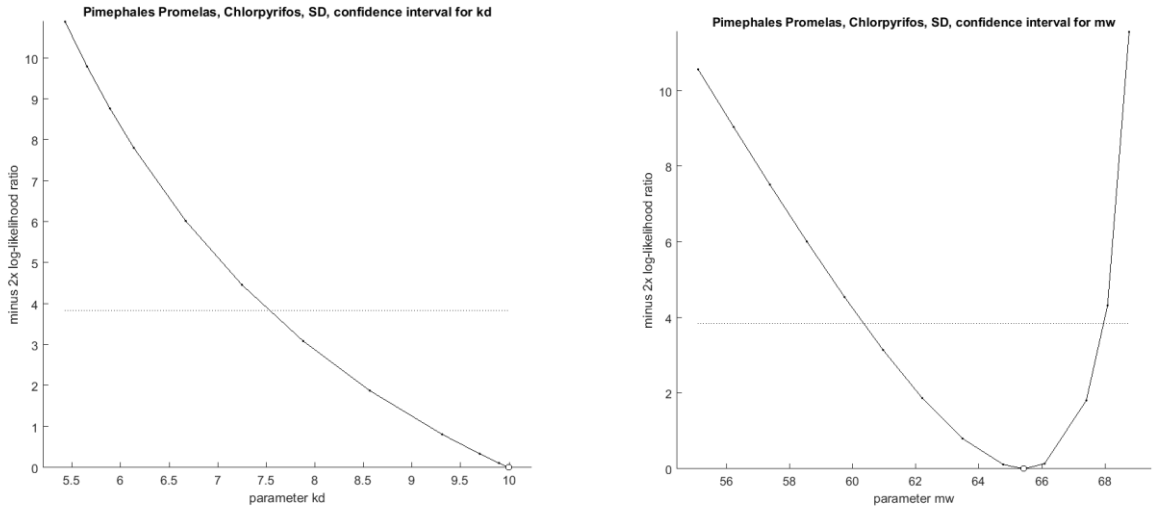

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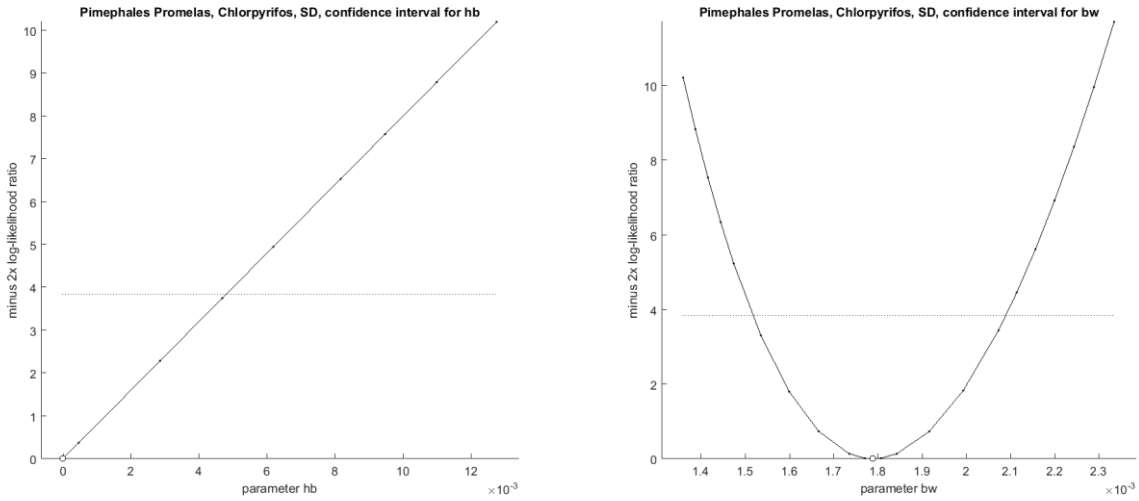

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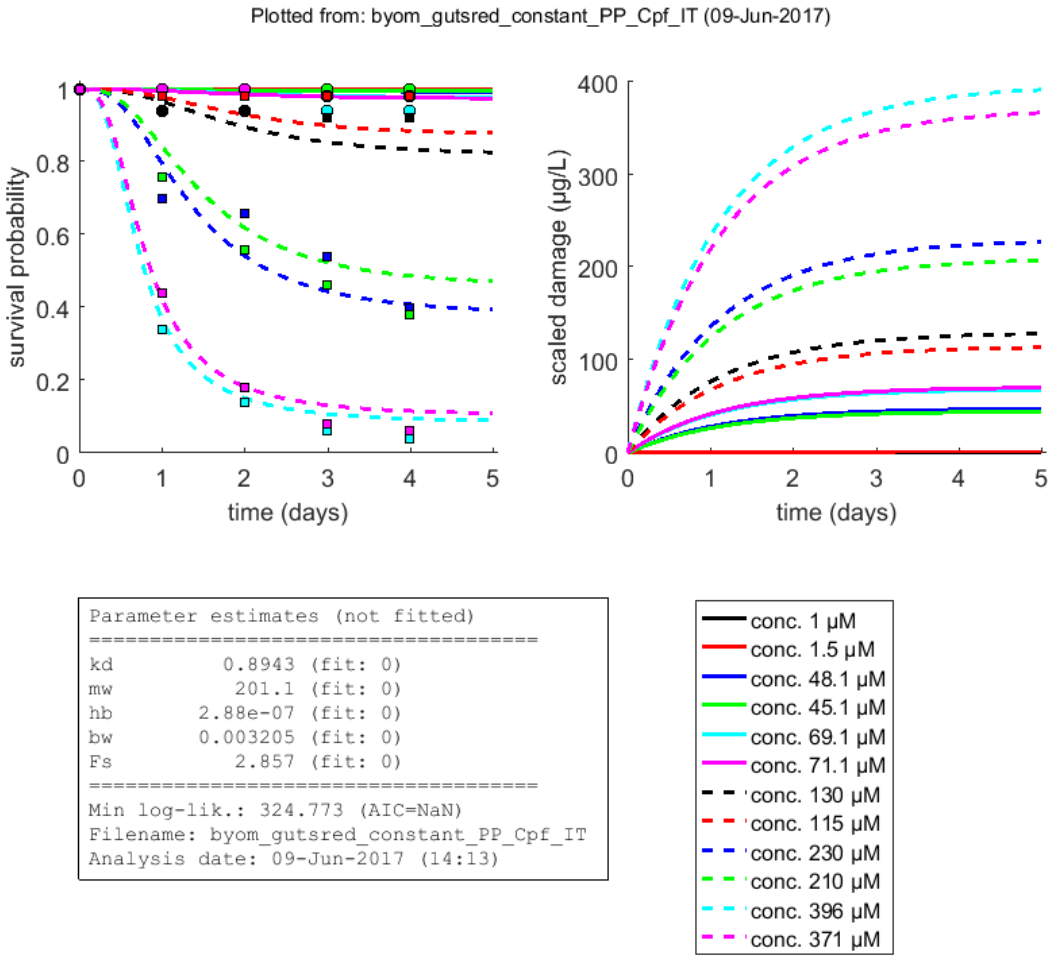

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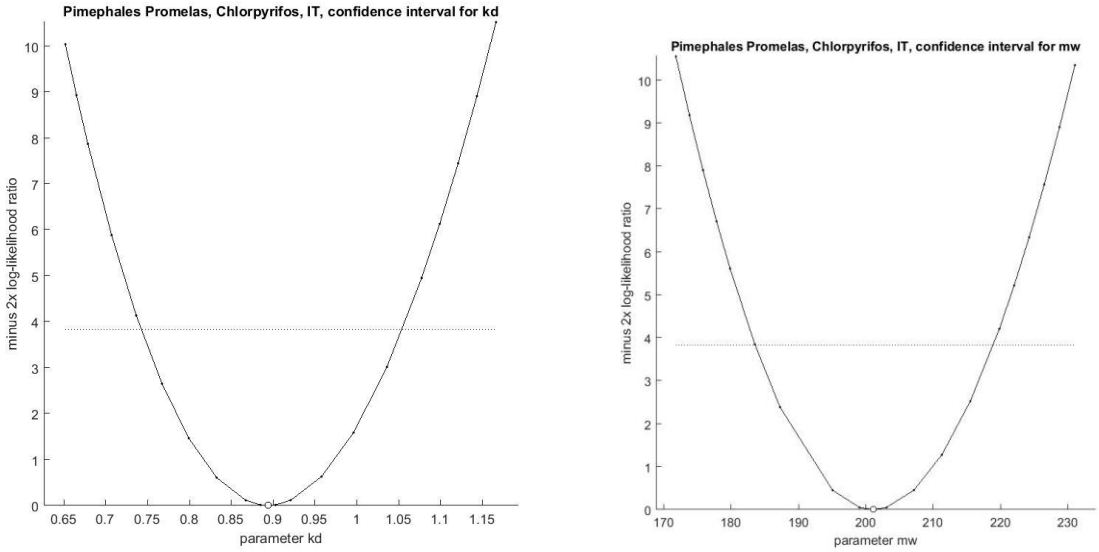

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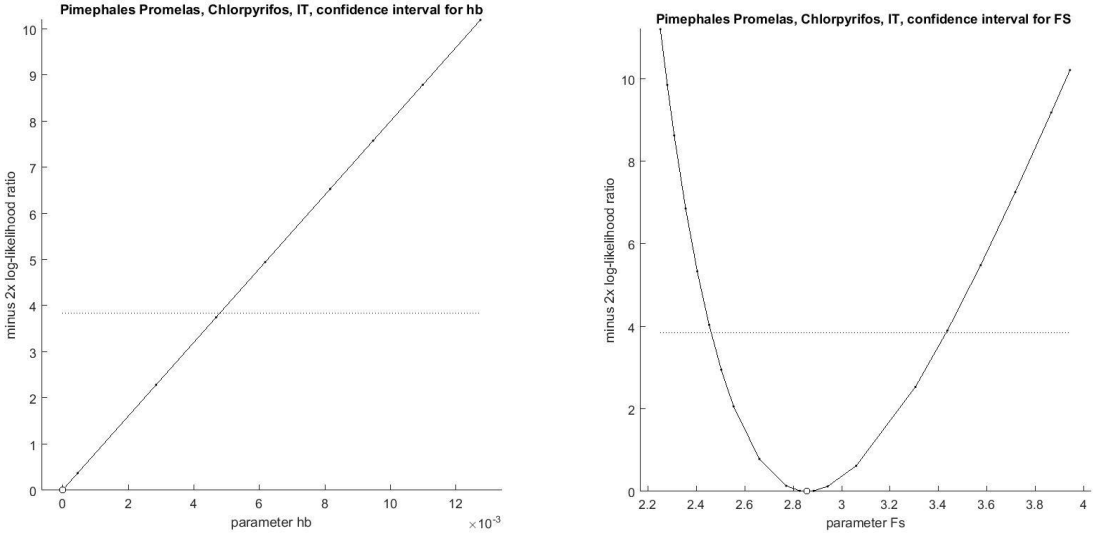

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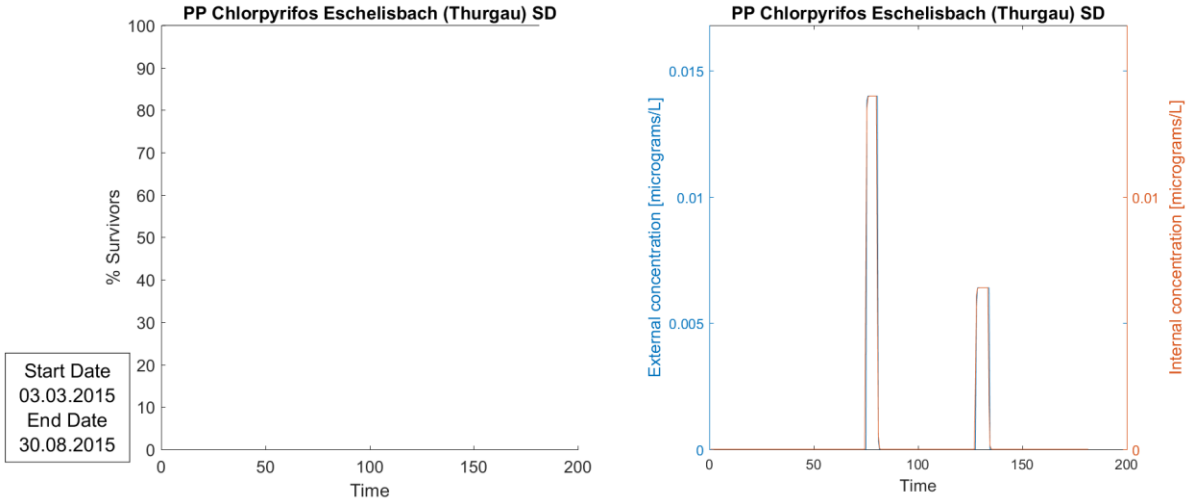

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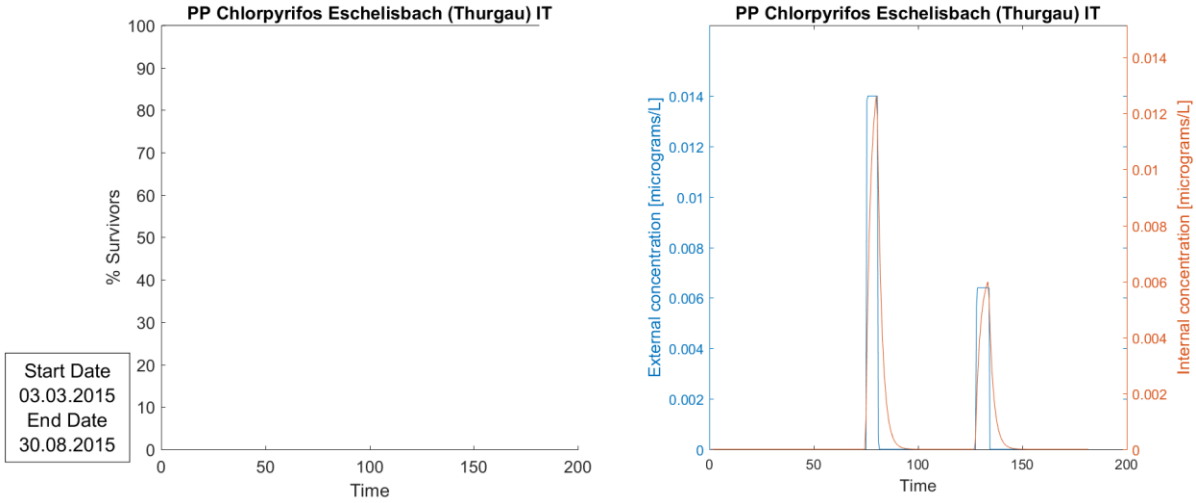

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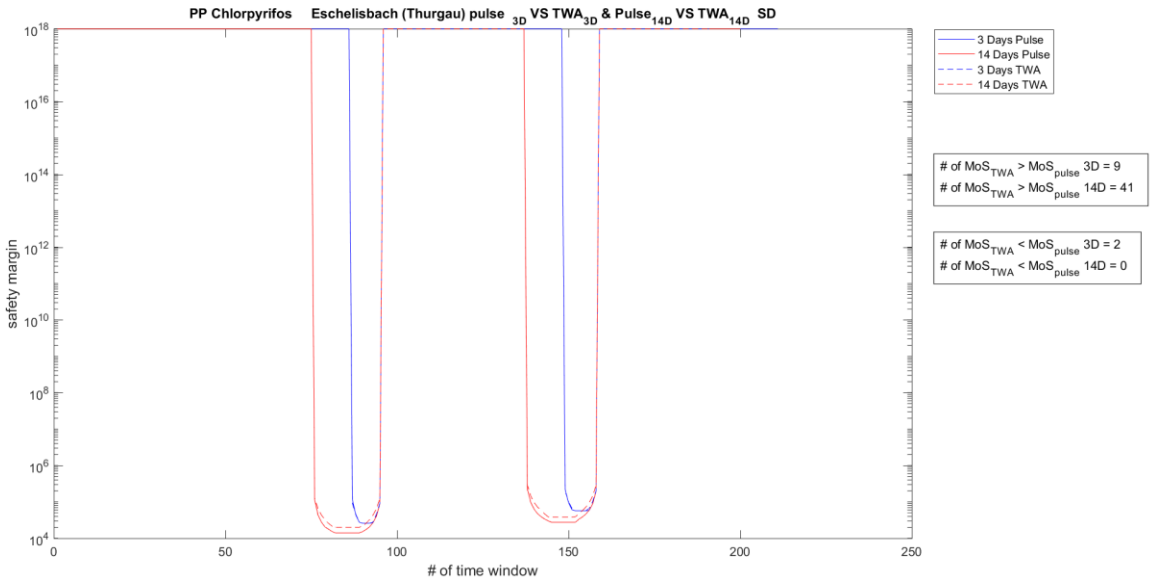

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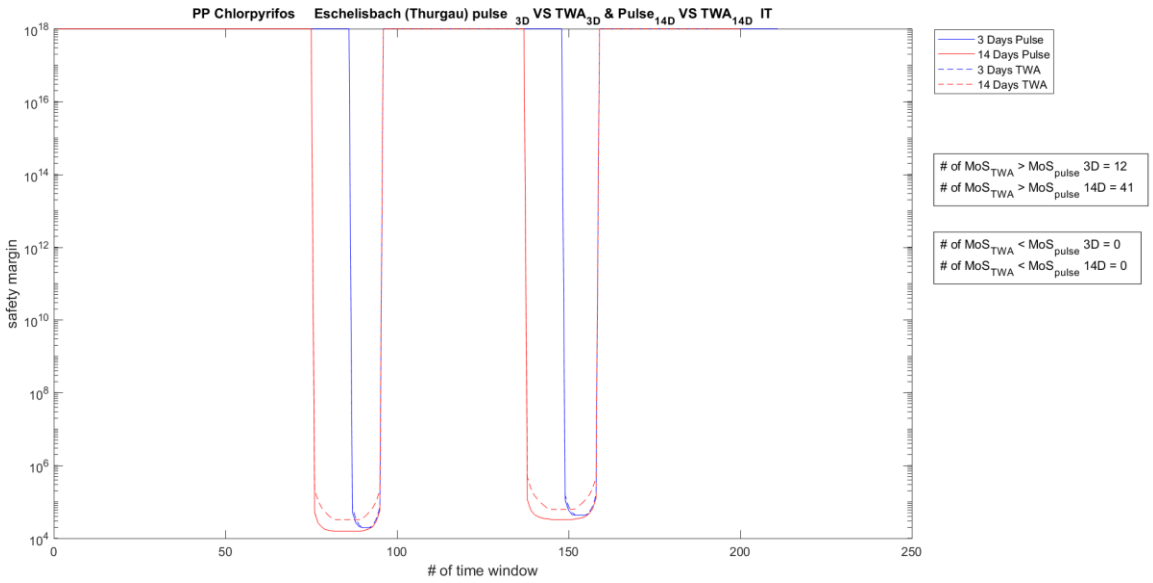

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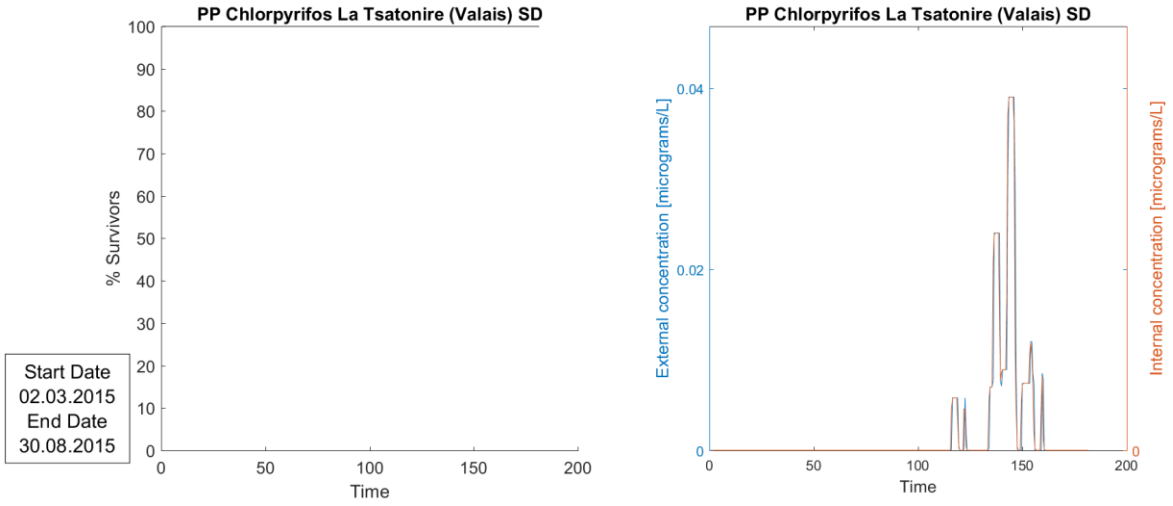

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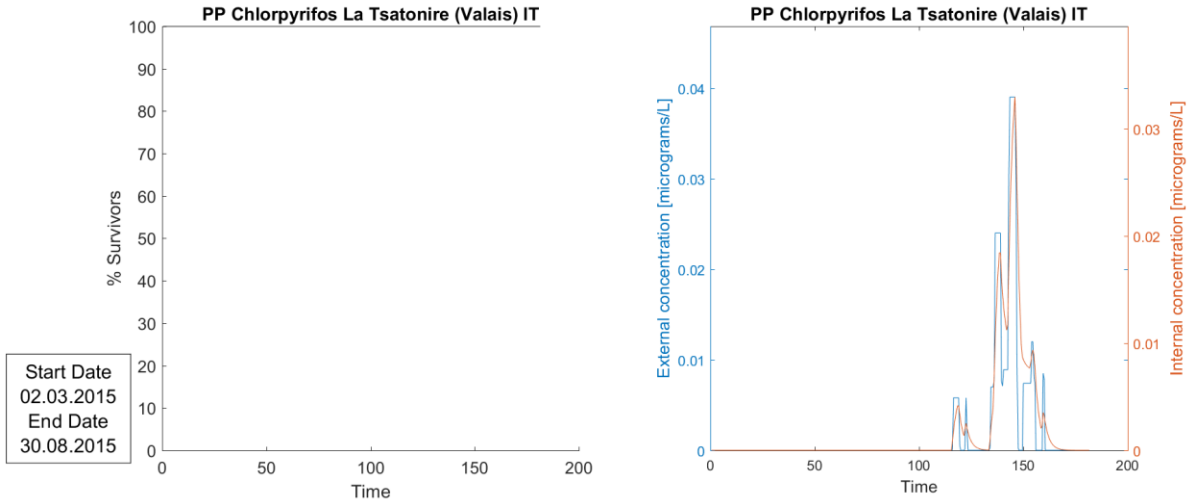

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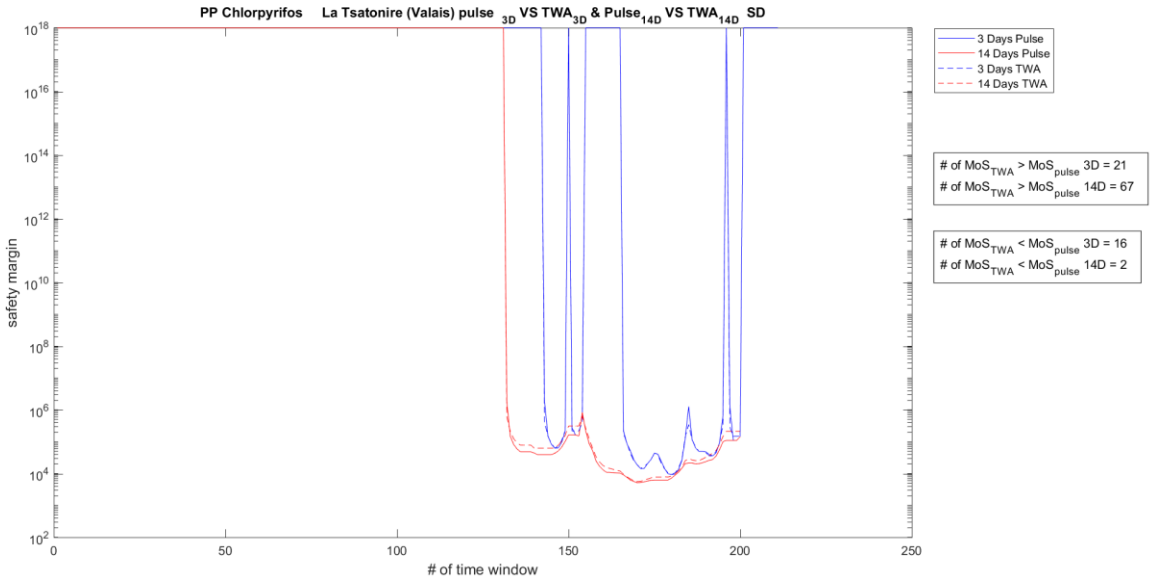

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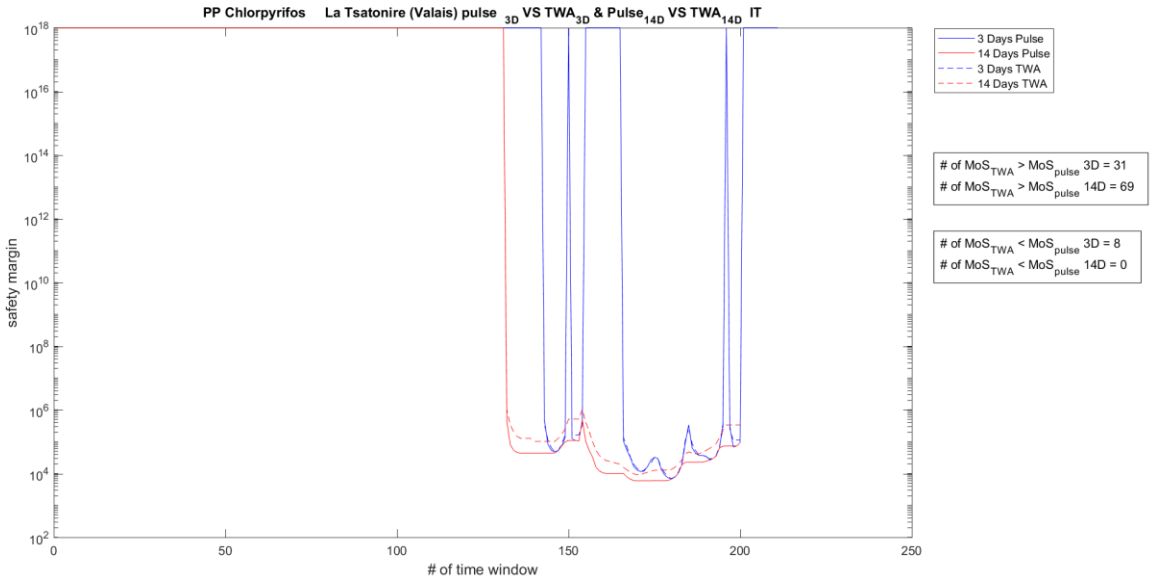

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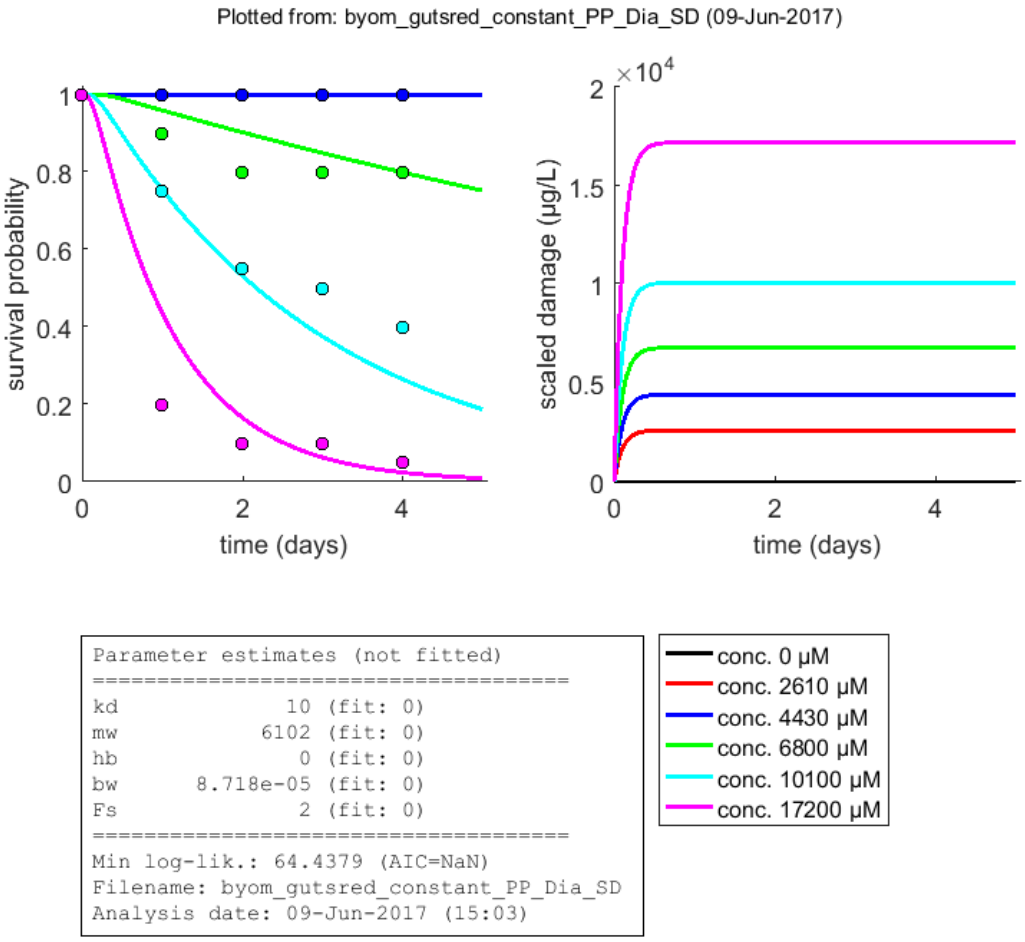

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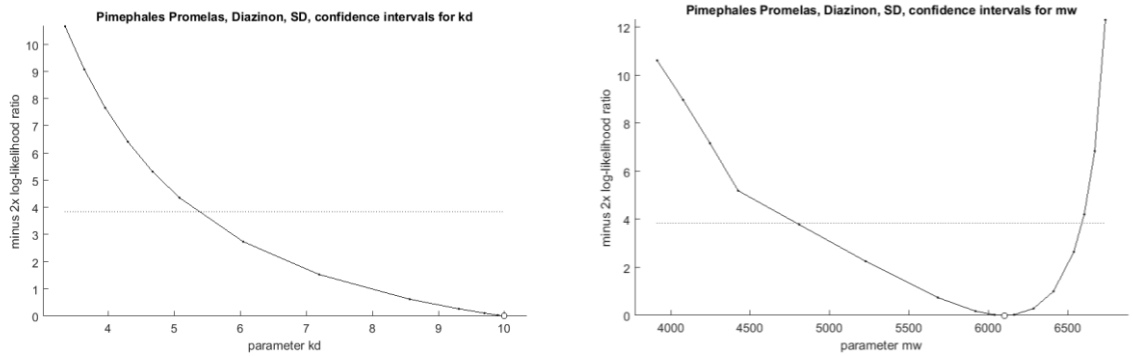

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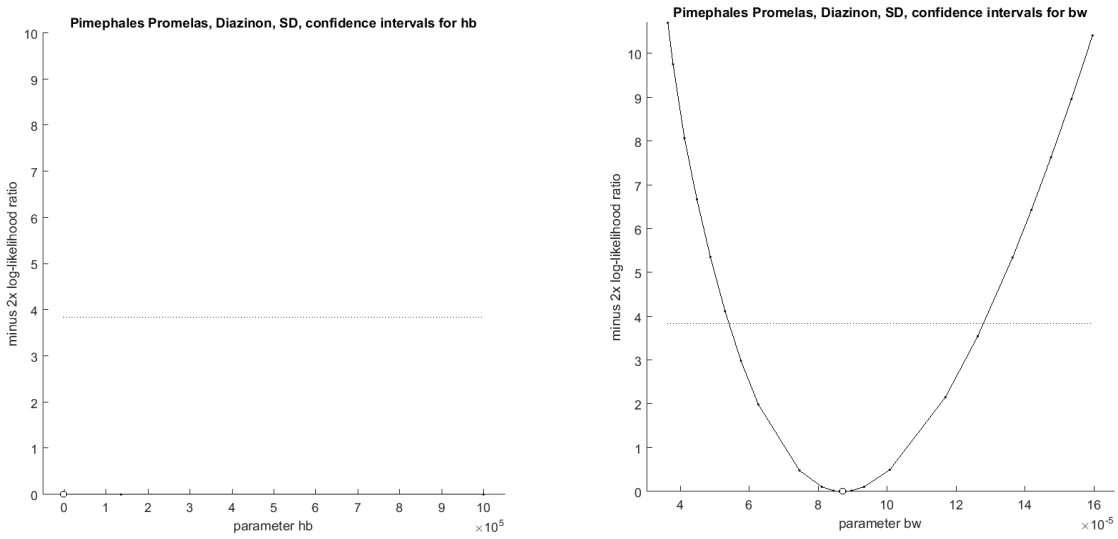

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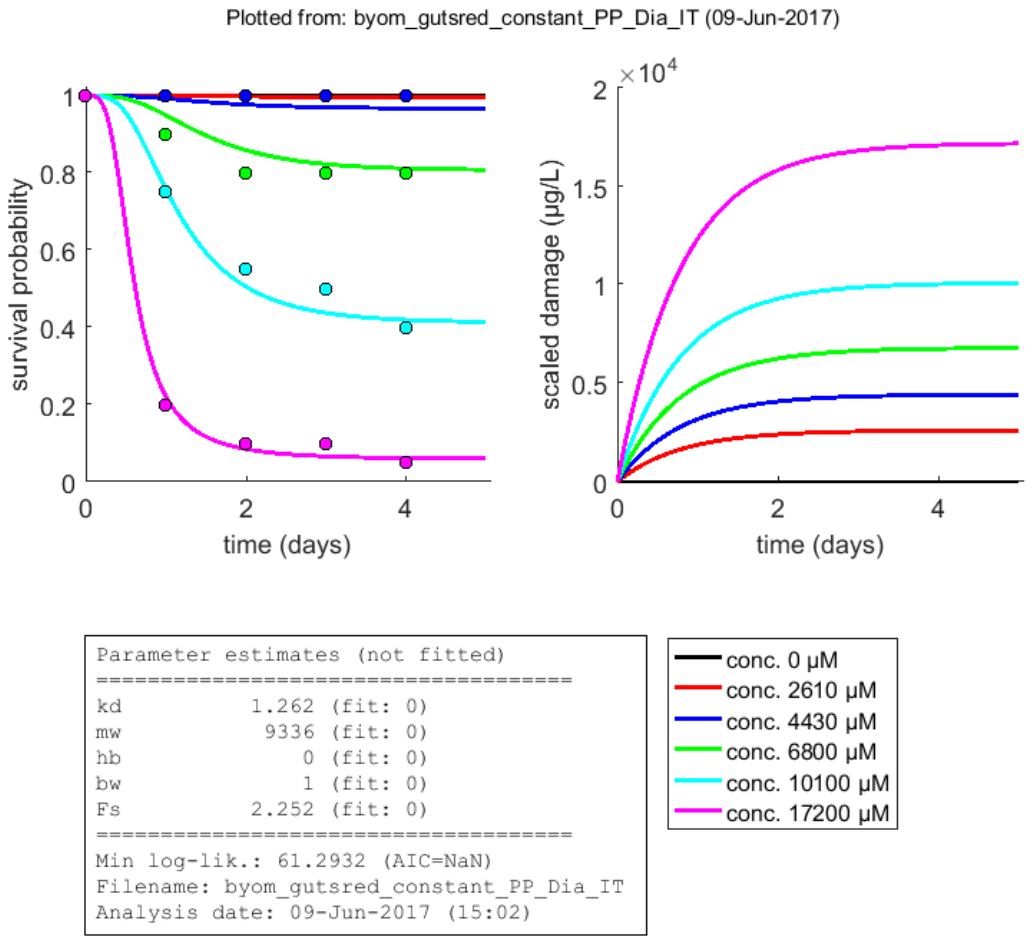

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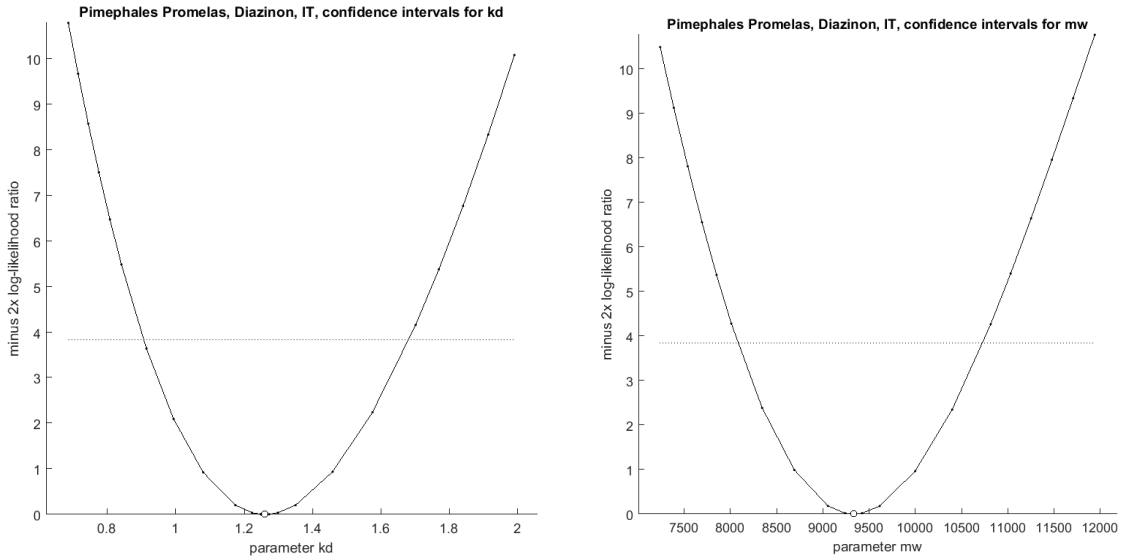

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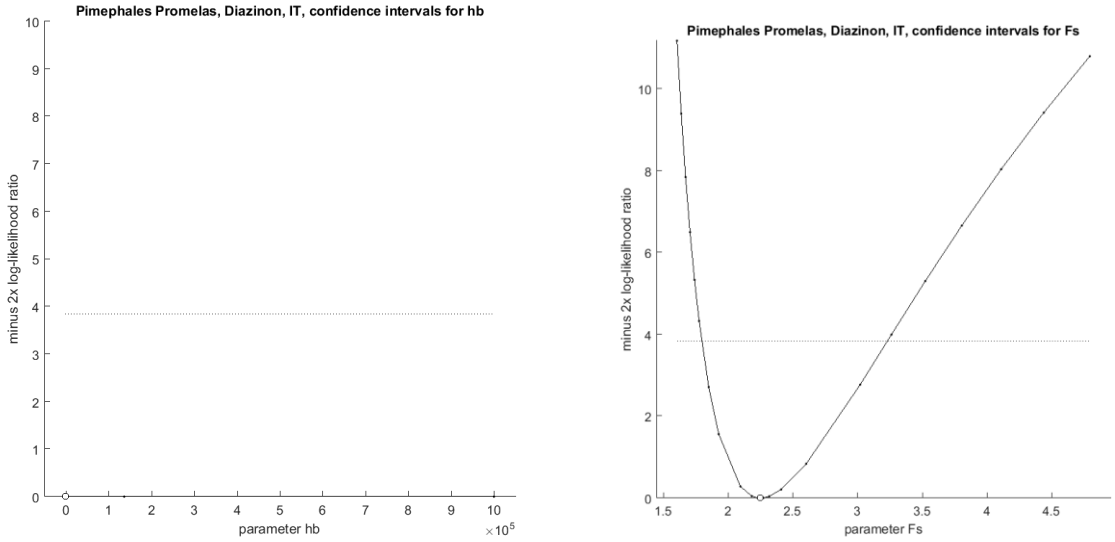

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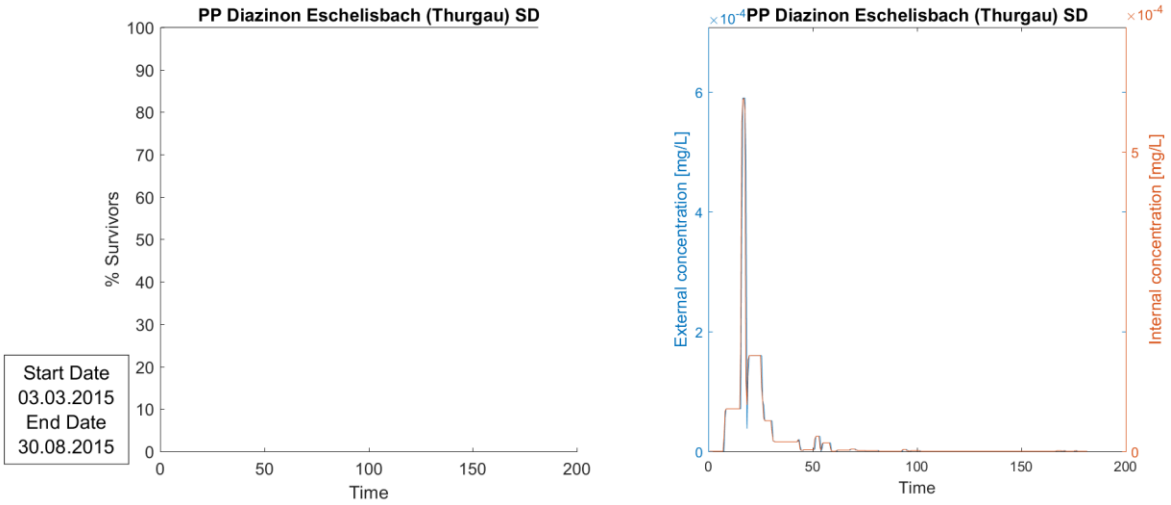

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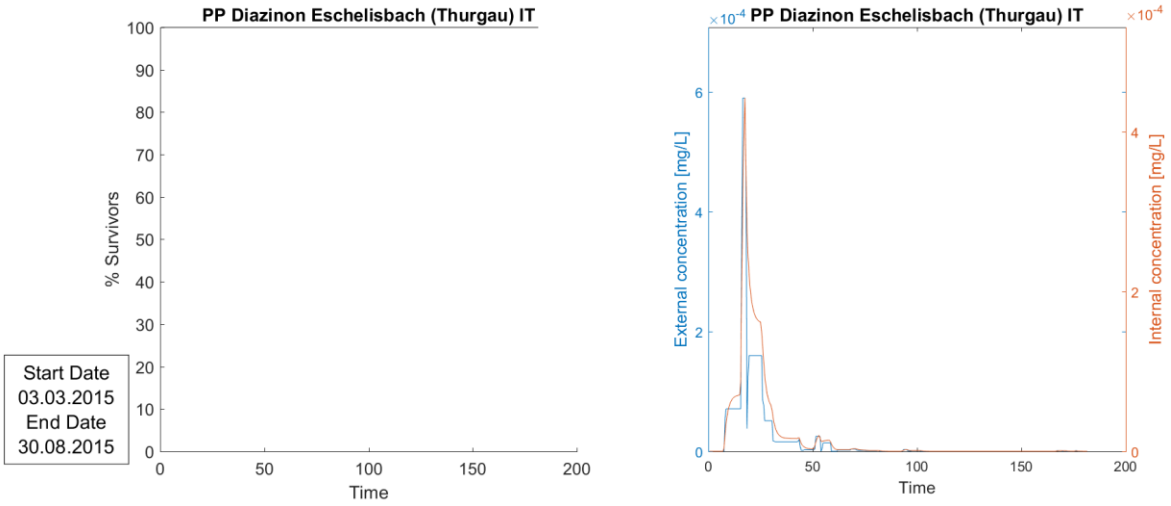

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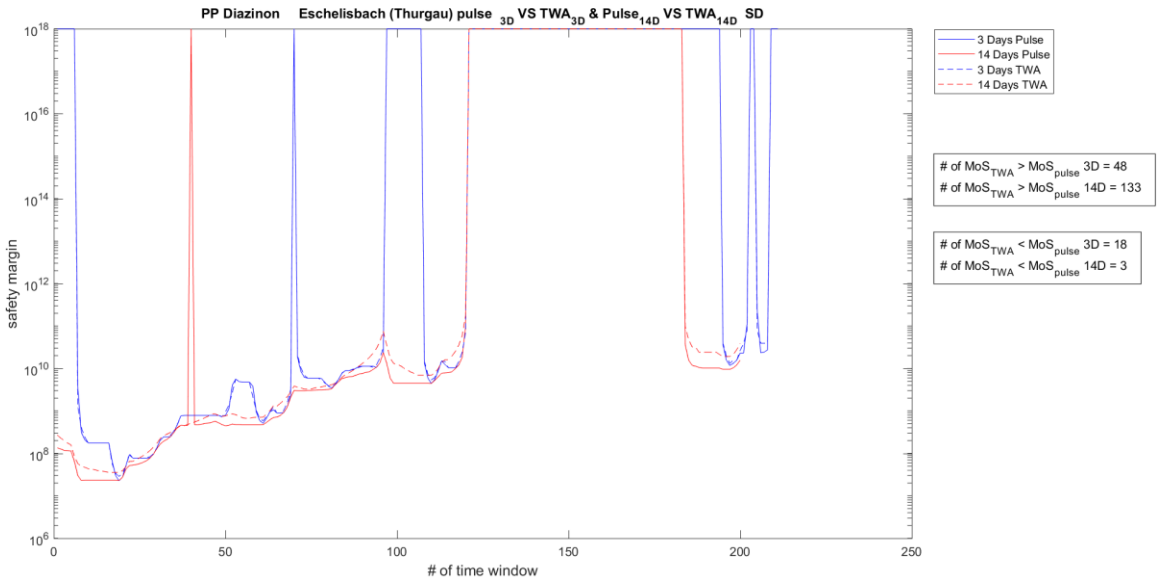

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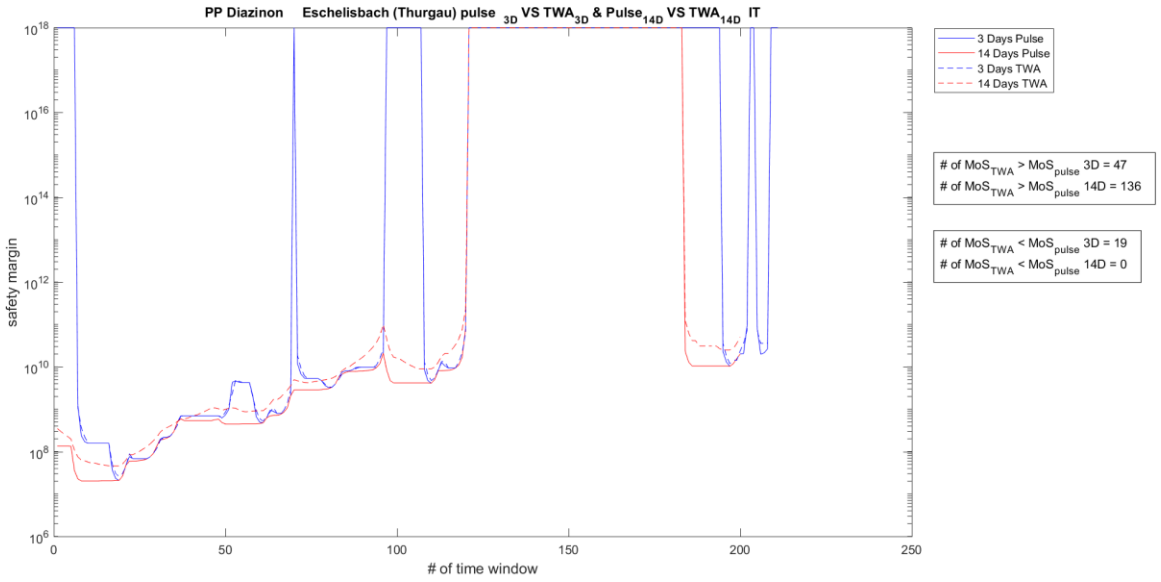

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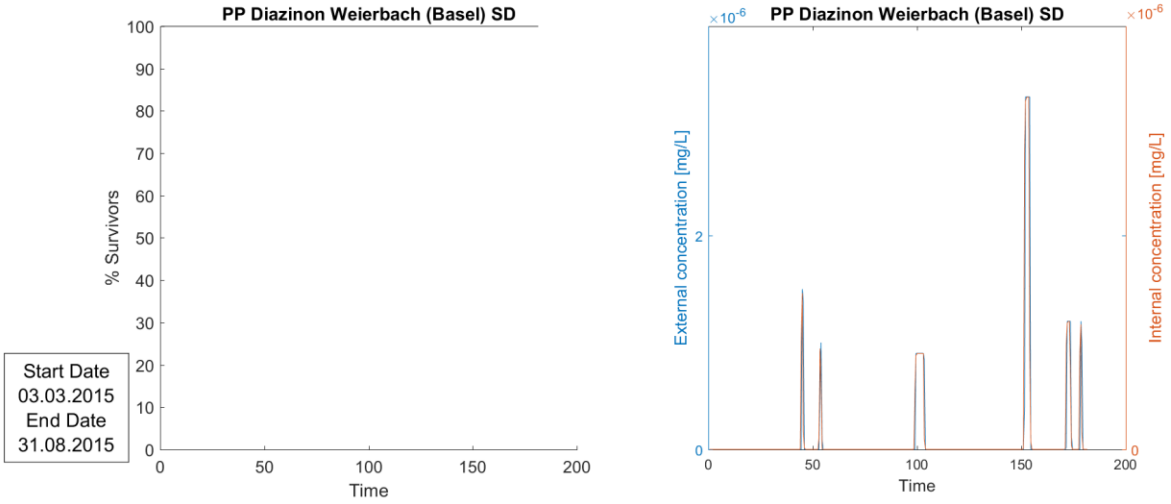

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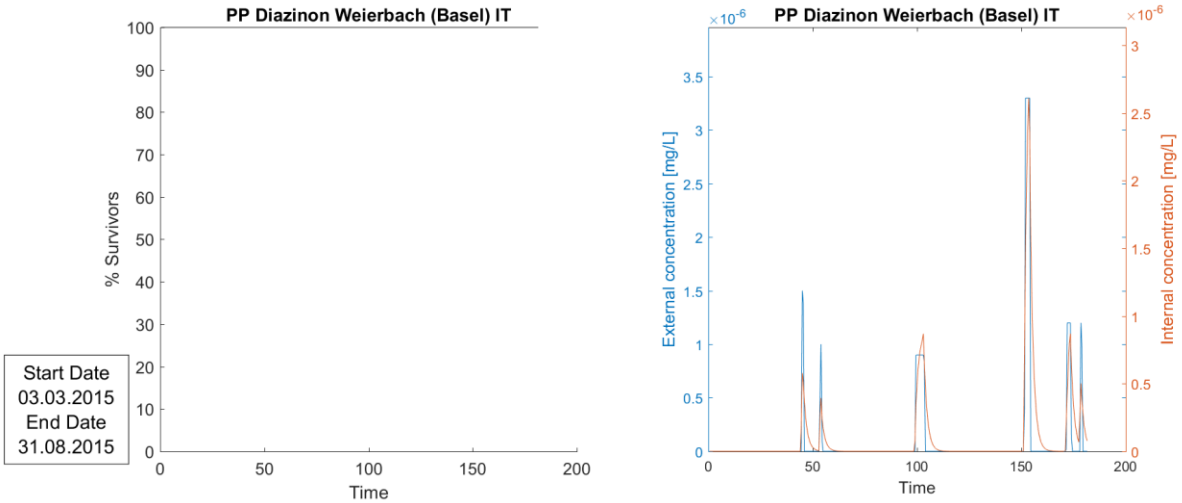

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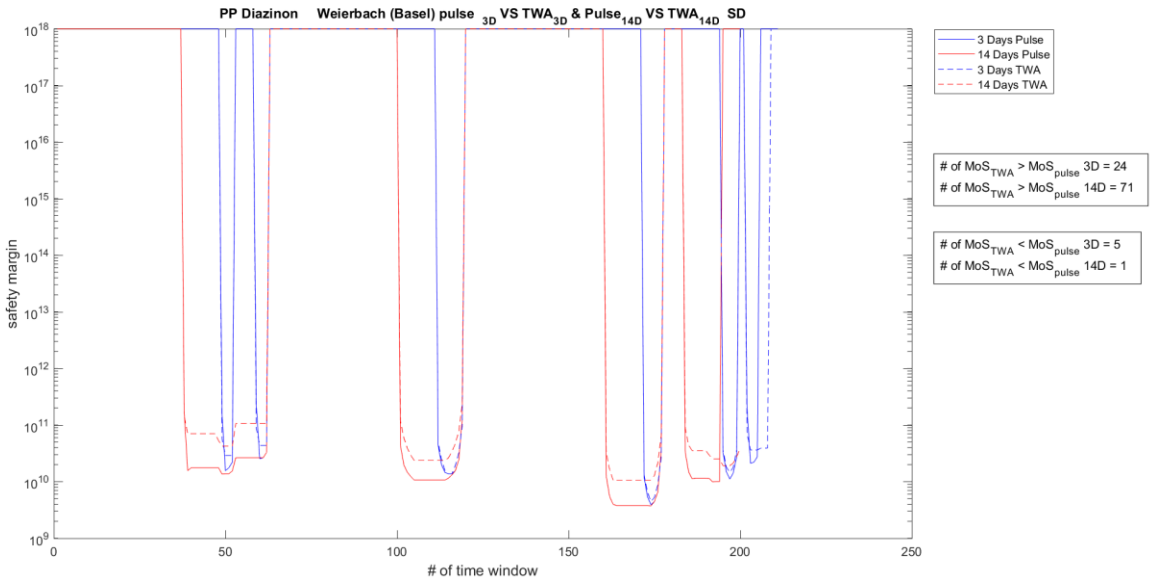

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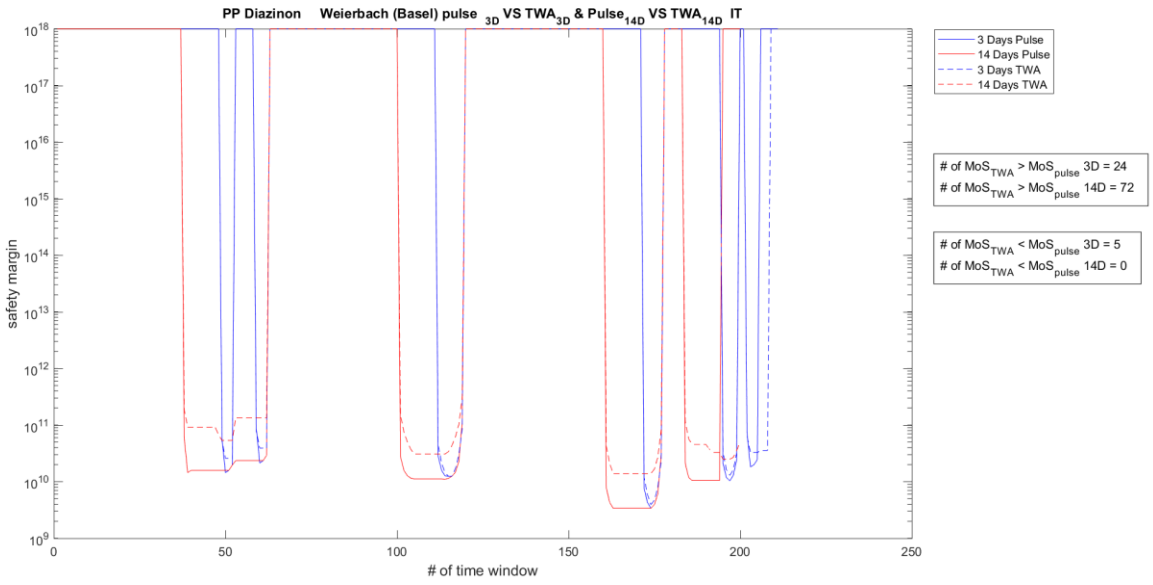

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Final Report

Simulation based assessment of Haber's law using modelling approaches (TKTD, GUTS)

- *Daphnia magna*

Author: **Roland Kuhl**
Dr. Elke Zimmer

Study Completion Date: August 18, 2017

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Project Staff:

Test Facility Management:
Dr. Melanie Lichtenberger

Study Director:
Roland Kuhl

1.2 Archiving

The following documents and materials will be archived for the at least 3 years:

- All electronic raw data
- the electronic final report
- any electronic final report amendment or any revised final report

Following the date on which the final report at

ibacon GmbH
Germany

After the archiving period, all raw data or material relating to the study will be discarded without the Sponsor's prior written consent.

1.3 Final Report Approval

Study Director: Dr. Elke Zimmer für Roland Kuhl

i.A. E. Zimmer

date: 31.08.2017

Test Facility Management: Dr. Melanie Lichtenberger

date: August 31, 2017

2. Materials and Methods

2.1 Data used for Modelling

The data on which the model was built were published in Agatz (2013). Data from two reproduction studies which were conducted at different food levels were used. In the first test, 0.15 and 12 mg/L Imidacloprid were tested at a standard food level as described in the OECD 211. In the second test 0.15, 0.4, 1.3, 4 and 12 mg/L Imidacloprid were tested at an approximate 70% food level. Both tests were conducted with 10 replicates per treatment with an exposure phase of 7 days, followed by a recovery phase in uncontaminated test medium. In the high food level test, the recovery phase lasted 30 days, in the low food test it lasted 27 days. During the test, body size, day of first reproduction, reproduction per female and survival was observed frequently.

2.2 Definitions

Margin of Safety: The margin of safety is the value with which a concentration is multiplied to lead to 50% effect in the simulation (MoS) for the 14d simulations and to 10% effect in the 3d simulations.

Time window: A 3 or 14 day timespan for which the time weighted average (TWA) of all measured concentrations was calculated. Since two samplings were performed each day, two timeframes with the same start day were calculated. For the 3 day simulations, 211 time windows were used, for the 14 day simulations, 200 time windows.

Underestimation: A margin of safety is considered as underestimation if the estimated margin of safety for a specific time-window using TWA is lower than the respective margin of safety from the real exposure profile.

2.3 Model equations for *Daphnia magna*:

Maintenance Rate Coefficient: $kM = rB * 3 * (1+g)/g$

Energy Conductance: $v = Lm * kM * g$

Stress Factor: $s = (1/cT)*\max(0,cV-c0)$

Feeding Rate Stress $f = f*\max(0,1-s)$

Change in Scaled Energy Density: $de = (f-e)*v/L$

Change in Length: $dL = (kM*g/(3*(e+g))) * (e*v/(kM*g) - L)$

Reproduction rate: $R = (Rm/(Lm^3-Lp^3))*((v*(L^2)/kM + L^3)*e/(e+g) - Lp^3)$

Change in internal Concentration: $dcV = ke*(Lm/L)*(c-cV) - cV*(3/L)*dL$

Hazard Rate: $h = b * \max(0,cV-c0s)$

Change in Survival: $dS = -(h + h0)* S$

Note: since we did not model survival, the equations for hazard rate and survival are not used and just added here for completeness.

2.4 Parameters for *Daphnia magna*:

ke:	elimination rate constant (d-1)
c0:	no-effect concentration sub-lethal (mg/L)
cT:	tolerance concentration (mg/L)
c0s:	no-effect concentration survival (mg/L)
b:	killing rate (1/(d * mg/L))
L0:	initial body length (mm)
Lp:	length at puberty (mm)
Lm:	maximum length (mm)
rB:	von Bertalanffy growth rate (d-1)
Rm:	maximum reproduction rate (eggs/d)
RmLF:	maximum reproduction rate for the low food level (eggs/d)
g:	energy-investment ratio (-)
f:	scaled functional response (-)
fLF:	scaled functional response for the low food level (-)
h0:	background hazard rate (d-1)

2.5 Parameter Estimation and Simulations for *Daphnia magna*

Test Items:	Imidacloprid	
Test Species:	*Daphnia magna*	
Software:	Matlab R2016a 64-bit version 9.0.0.341360 (February 11, 2016) Matlab 2017a 64-bit version 9.2.0.556.344 (March 27, 2017) BYOM for Matlab version 4.01 (May 12, 2017) DEBtox version 2.0 (April 28, 2017)	
Solver:	ode45	
Parameter Estimation Settings:	Relative Tolerance:	$1x10^{-4}$
	Absolute Tolerance:	$1x10^{-7}$
	Initial Step Size:	Max(t)/1000
	Maximum Step Size:	Max(t)/100
	where t is the time vector of the data	
Parameter Estimation:	We used a stepwise approach for parameter estimation. At first, the basic DEBtox parameters were estimated using control data of the high food level for body length over time, cumulative number of offspring were used to estimate the parameters L0, Lp, Lm, rB, Rm and h0. Then, the control data from the low food level experiment were added to estimate and the food level for this experiment fLF and a reproduction rate RmLF. It is known that daphnids produce	

smaller offspring at low food conditions, so it is not surprising that we needed to estimate a separate reproduction rate for the lower food level. All estimated parameters were then fixed and the toxicological data from both experiments were included to fit the parameters ke, c0 and cT. Since no significant mortality occurred in the experiments, c0s was fixed to 10000 and b was fixed to 0.00001.

After 25 days, survival and reproduction in the control (and the treatments) decreased substantially, presumably due to ageing. Since the DEBtox model does not include an ageing module, it was decided to only use the data until day 25.

The effects on the daphnids in the higher food concentrations deviate from the effects on the daphnids in the lower food concentration. The maximum growth achieved in the control is the same for both food levels, which forces the DEBtox model to find a similar value for the functional response for both food levels.

The first tries to properly fit the effects on the daphnids did not succeed. The reduction in ultimate size and the decline in reproduction after 25 days could not be replicated. Several things were tested to achieve a better fit:

- Introduction of a separate f for the first week of the test, as feeding was different there. However, due to the smaller size of the animals, the food availability should proportionally be the same.
- Combination of several stress factors *e.g.* maintenance, growth and reproduction in addition to feeding inhibition
- Introduction of a damage module with slower damage repair as it seemed the damage is still present after the exposure phase.

All of the steps above did not improve the fit significantly. Therefore, it was decided to go for the simplest approach and to only include an effect on feeding, which fits the observed patterns in general in both growth and reproduction (see Figure 1 and Figure 2). Alternatively, a full DEB model could be tested with changes in kappa (κ) to improve the fit.

Simulations:

With the calculated parameters, simulations were performed with 3d and 14d TWA time windows with growth and reproduction as endpoints.

The Margin of Safety (MoS) which leads to 50 % reduction in the respective endpoints for the 14d simulations and 15% reduction for the 3d simulations was calculated.

The parameters shown in table 1 were used for the simulations. As a default, a value of f=1 was used.

Due to the short lifespan of daphnids, a simulation for effects over the whole time period was considered unrealistic and thus not performed.

As a maximum safety margin, we assumed $1x10^{19}$.

3. Results for *Daphnia magna*

Table 1. Parameters used for the Simulations with *Daphnia magna*

Parameter	Value	Unit	Confidence Intervals	Description
ke*	98.72	d-1	-	elimination rate constant
c0	1.712×10^{-7}	nM/L	0 - 986.4	no-effect concentration sub-lethal
cT	8.848×10^{4}	nM/L	8.354×10^{4} - 9.477×10^{4}	tolerance concentration
c0s	3.911×10^{7}	nM/L	-	no-effect concentration survival
b	0.0391	1/d nM/L	-	killing rate
L0	0.9205	mm	0.8045 - 1.041	initial body length
Lp	2.449	mm	2.276 - 2.595	length at puberty
Lm	4.204	mm	4.049 - 4.424	maximum length
rB	0.09699	d-1	0.08265 - 0.1101	von Bertalanffy growth rate
Rm	23.76	eggs/d	21.05 - 27.57	maximum reproduction rate
RmLF	11.67	eggs/d	9.846 - 14.22	maximum reproduction rate for the low food level
g*	0.422	-	-	energy-investment ratio
f	1	-	-	scaled functional response
fLF	0.985	-	0.9459 - 1	scaled functional response for the low food level
h0	0.004241	d-1	0.0007152 - 0.01307	background hazard rate

*ke and g were not fitted in the final parameter estimation since earlier estimations showed that both parameters trended towards 100 or 0. Therefore, in case of ke the value of an earlier parameter estimation was used, while for g the standard value for *Daphnia magna* from the BYOM was used.

3.1 Simulation results

Simulation Results:

The graphs show the safety margin as 1×10^{18} when either the concentration was zero, or when the concentration times the safety factor was still lower that the no-effect concentration.
For the 3 day simulations, no effects were observed.
The results for the 14 day simulations are summarized below (see also Table 5).

For Eschelisbach, the TWA simulations overestimated the effects for length in 61% of the time; effects on offspring were more often underestimated. A similar effect pattern can be seen for La Tsatonire, where effects on length are overestimated in 51% of the time.

For Weierbach, again the effects on lengths were overestimated by the TWA approach, while effects on reproduction are predicted almost equally well with both approaches.

As expected, due to the low concentration in the exposure profile (max. 40 ng/L), only slight effects were found in the simulations with the lowest MoS of 1.5E+08.

Table 2. The 3 most toxic time windows leading to the lowest margins of safety for 3d simulations

Daphnia magna

Chemical and Location	Lowest Margin of Safety 3d TWA 1st	d	2nd	d	3rd	d	3d pulse 1st	d	2nd	d	3rd	d
Imidacloprid							**Length**					
Eschelisbach	1.0E+18	1	1.0E+18	2	1.0E+18	3	1.0E+18	1	1.0E+18	2	1.0E+18	3
La Tsatonire	1.0E+18	1	1.0E+18	2	1.0E+18	3	1.0E+18	1	1.0E+18	2	1.0E+18	3
Weierbach	1.0E+18	1	1.0E+18	2	1.0E+18	3	1.0E+18	1	1.0E+18	2	1.0E+18	3
Imidacloprid							**Offspring**					
Eschelisbach	1.0E+18	1	1.0E+18	2	1.0E+18	3	1.0E+18	1	1.0E+18	2	1.0E+18	3
La Tsatonire	1.0E+18	1	1.0E+18	2	1.0E+18	3	1.0E+18	1	1.0E+18	2	1.0E+18	3
Weierbach	1.0E+18	1	1.0E+18	2	1.0E+18	3	1.0E+18	1	1.0E+18	2	1.0E+18	3
	min: 1.0E+18 max: 1.0E+18 mean: 1.0E+18						min: 1.0E+18 max: 1.0E+18 mean: 1.0E+18					

Table 3. The 3 most toxic time windows leading to the lowest margins of safety for 14d simulations

Chemical and Location	Lowest Margin of Safety 14d TWA 1st	d	2nd	d	3rd	d	14d pulse 1st	d	2nd	d	3rd	d
Imidacloprid							**Length**					
Eschelisbach	3,9E+09	53	3,9E+09	57	3,9E+09	54	8,8E+09	49,5	9,1E+09	102	9,1E+09	103
La Tsatonire	1,5E+10	142	1,5E+10	141	1,6E+10	139	2,4E+10	141	2,4E+10	140	2,4E+10	142
Weierbach	7,2E+09	21,5	7,2E+09	22,5	7,2E+09	23	1,6E+10	22,5	1,6E+10	23	1,6E+10	24
Imidacloprid							**Offspring**					
Eschelisbach	1,9E+08	53	1,9E+08	54	1,9E+08	55	1,5E+08	51,5	1,6E+08	52,5	1,6E+08	53,0
La Tsatonire	7,2E+08	142	7,4E+08	141	7,6E+08	140	6,8E+08	141	6,8E+08	142	6,8E+08	140
Weierbach	3,1E+08	21,5	3,1E+08	22,5	3,1E+08	23	2,7E+08	24	2,7E+08	25	2,7E+08	25,5
	min: 1.9E+08 max: 1.6E+10 mean: 4.6E+09						Min: 1.5E+08 Max: 2.4E+10 Mean: 8.3E+09					

Table 4. Comparison of MoS of simulation results of *Daphnia magna* from 3d TWA calculations with pulse exposure results

Daphnia magna

Chemical and Location	Comparison of TWA simluation vs. real exposure scenario 3d TWA < 3d pulse		3d TWA > 3d	
Imidacloprid		**Length**		
Eschelisbach	0	0 %	0	0 %
La Tsatonire	0	0 %	0	0 %
Weierbach	0	0 %	0	0 %
Imidacloprid		**Reproduction**		
Eschelisbach	0	0 %	0	0 %
La Tsatonire	0	0 %	0	0 %
Weierbach	0	0 %	0	0 %

Table 5. Comparison of MoS of simulation results of *Daphnia magna* from 14d TWA calculations with pulse exposure results

Daphnia magna

Chemical and Location	Comparison of TWA simluation vs. real exposure scenario 14d TWA < 14d pulse		14d TWA > 14d	
Imidacloprid		**Length**		
Eschelisbach	121	61 %	56	23 %
La Tsatonire	102	51 %	5	3 %
Weierbach	123	62 %	20	10 %
Imidacloprid		**Reproduction**		
Eschelisbach	70	35 %	88	44 %
La Tsatonire	44	22 %	59	30 %
Weierbach	68	34 %	70	35 %

4. Discussion

- Both f values were very close to each other although the test aimed at a 70% food level for the low food condition
- The model was not able to reproduce the decline in reproduction after 25 days.
- The model was not able to reproduce the decrease in body size with increasing concentrations in the low food test.
- The model underestimated the reproduction in highest concentration of both food levels while overestimating the reproduction in the lower concentration of the high food level test as well as the 4 mg/L treatment of the low food level test. However, as the concentrations used for the simulations (max = 43 ng/L) are far below the lowest tested concentration (150000 ng/L) and the reproductive output after 14 days is used, the model predicts the reproduction for these data quite well (see Figure 1 and Figure 2).

5. References

Agatz A., Cole T.A., Preuss T.G., Zimmer E., Brown C.D. (2013). Feeding Inhibition Explains Effects of Imidacloprid on the Growth, Maturation, Reproduction, and Survival of Daphnia magna. Environmental Science & Technology 47: 2909-2917

Jager T., Albert C., Preuss T.G., Ashauer R. (2011). General unified threshold model of survival - a toxicokinetic-toxicodynamic framework for ecotoxicology. Environmental Science & Technology 45 (7): 2529–2540. DOI: 10.1021/es103092a

6. Distribution of the Final Report

Sponsor:	electronic copy
ibacon:	original

Appendix I: Tables and Figures

Parameters and Confidence Intervals for *Daphnia magna* and Imidacloprid

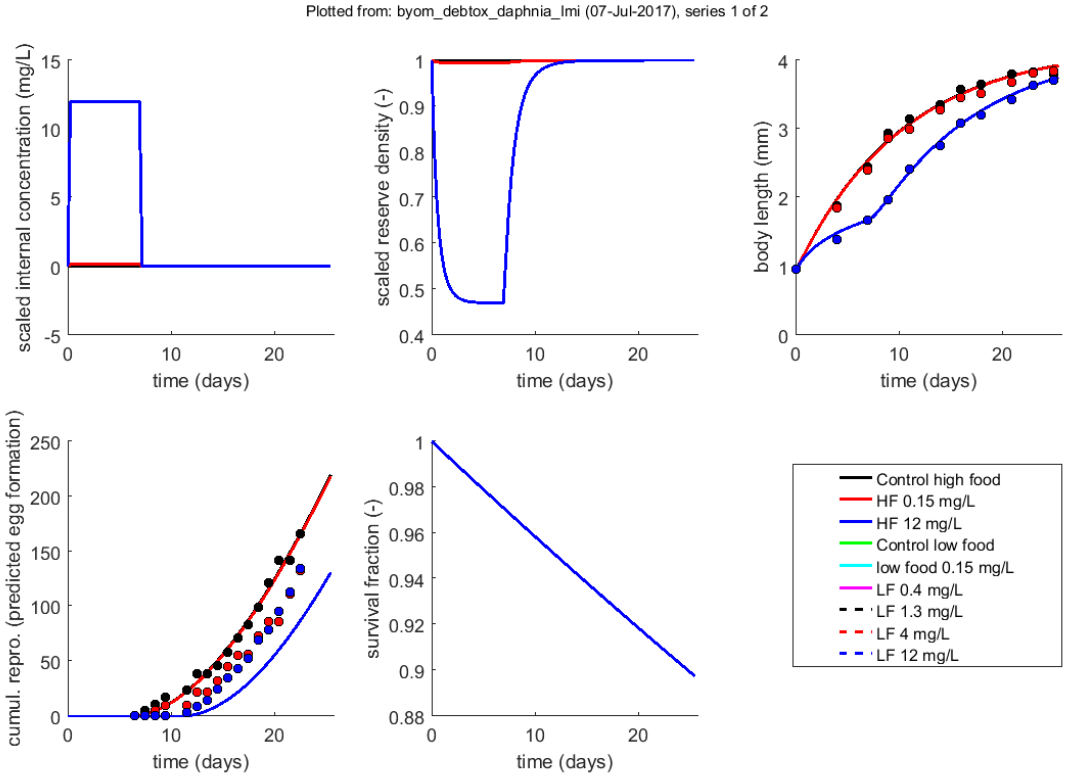

Figure 1. Graph for *Daphnia magna* high food level estimated parameters

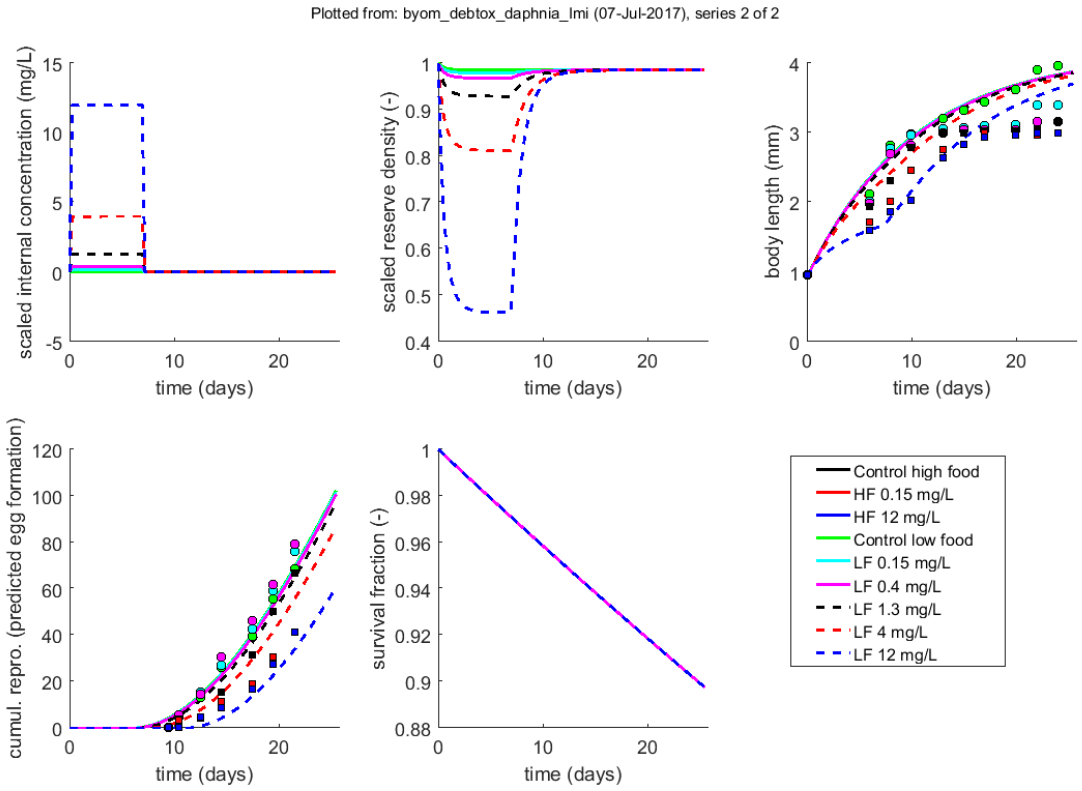

Figure 2. Graph for *Daphnia magna* low food level estimated parameters

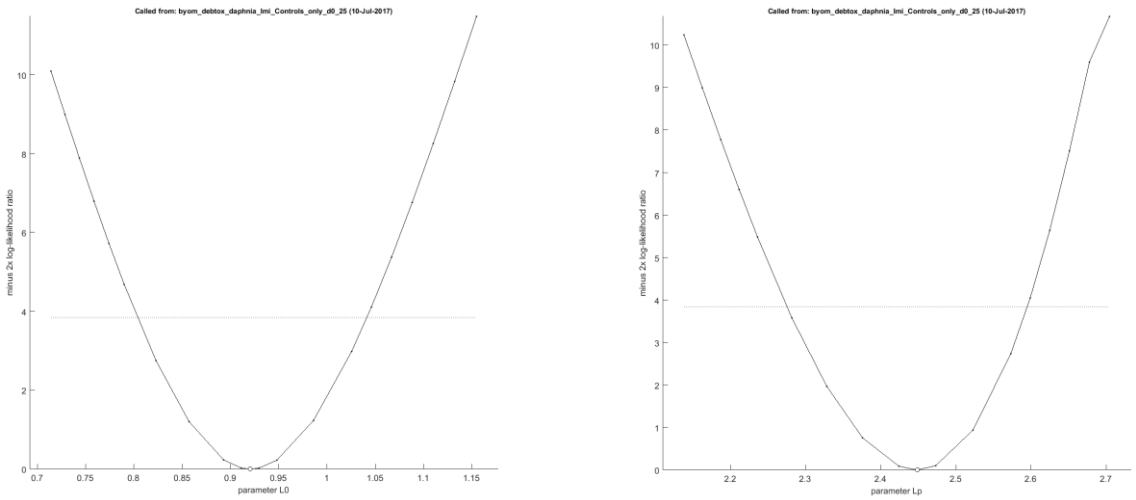

Figure 3. Confidence Intervals for *Daphnia magna* parameters L0 and Lp

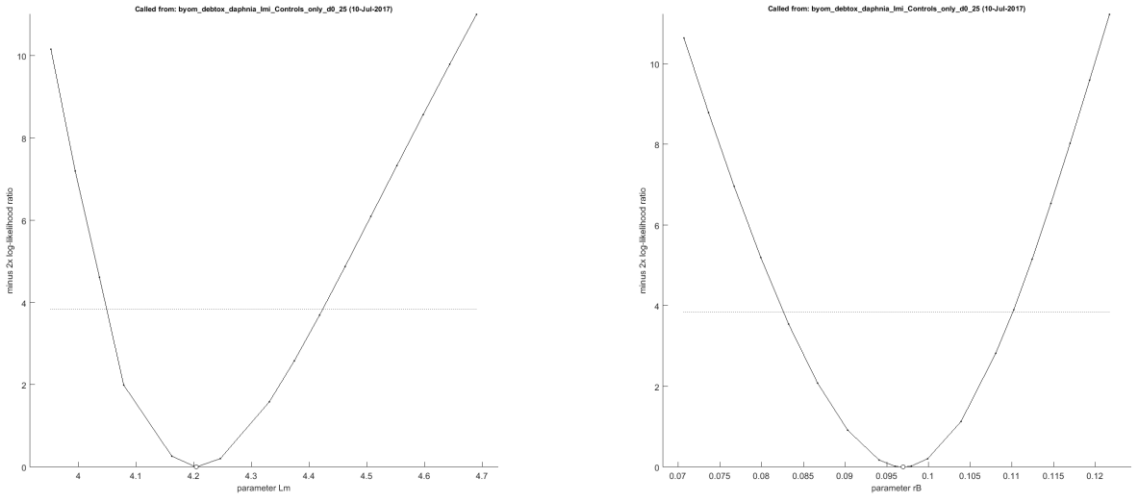

Figure 4. Confidence Intervals for *Daphnia magna* parameters Lm and rB

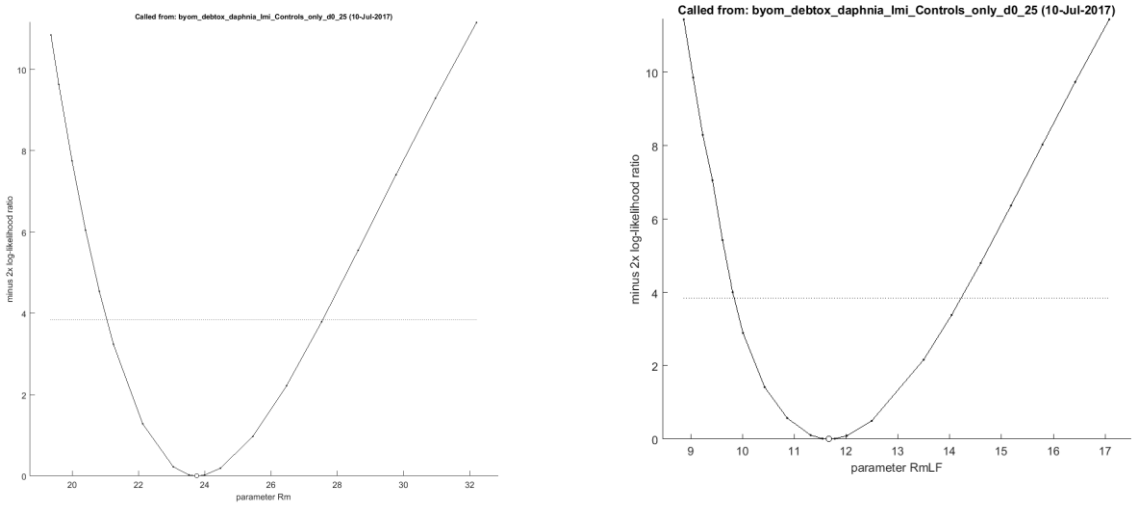

Figure 5. Confidence Intervals for *Daphnia magna* parameters Rm and RmLF

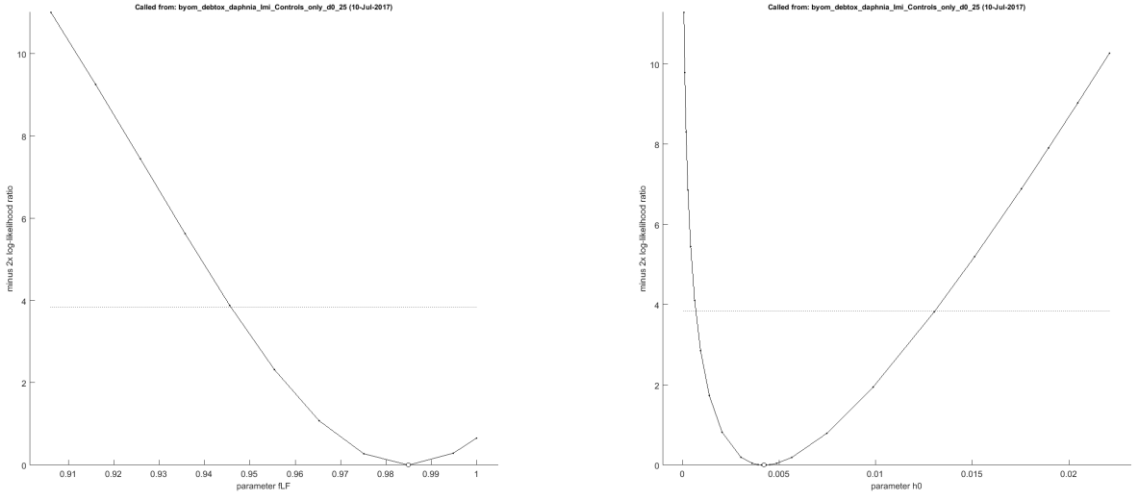

Figure 6. Confidence Intervals for *Daphnia magna* parameters fLF and h0

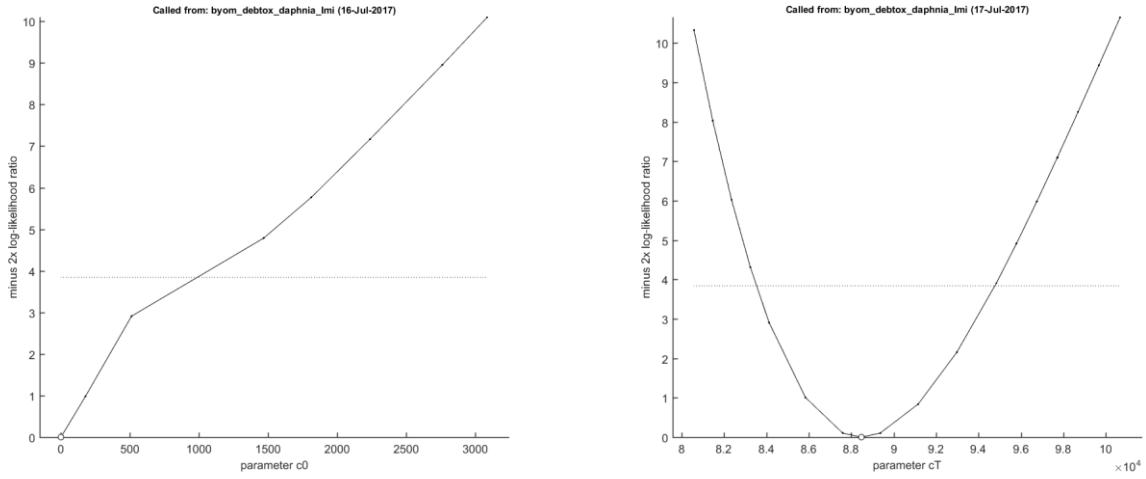

Figure 7. Confidence Intervals for *Daphnia magna* parameters c0 and cT

Plots for *Daphnia magna* and Imidacloprid

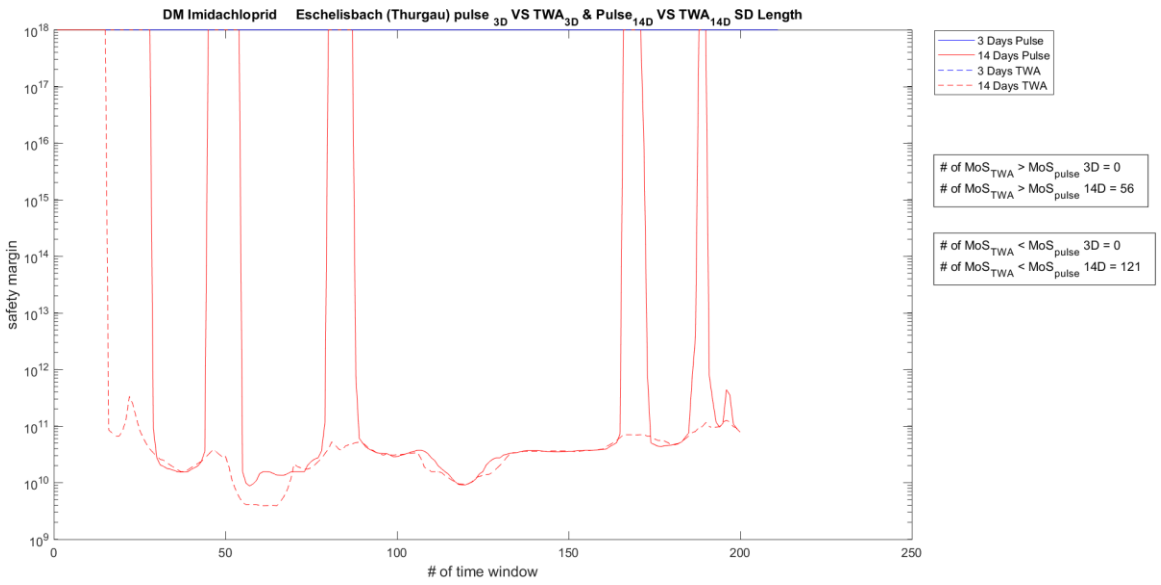

Figure 8. Margins of Safety of *Daphnia magna* for length, TWA and pulse, Eschelisbach

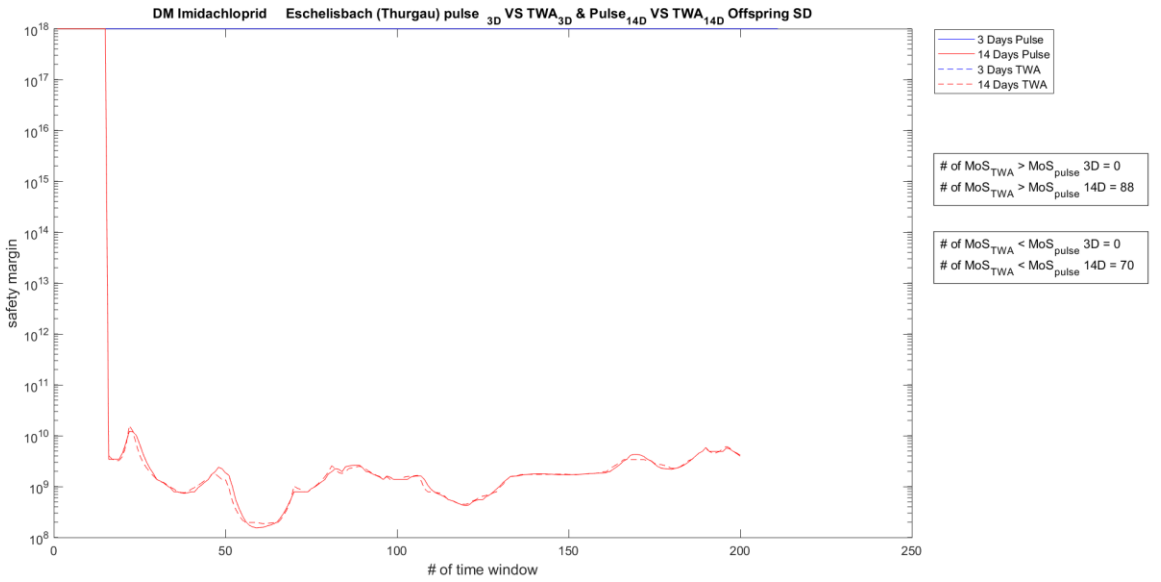

Figure 9. Margins of Safety of *Daphnia magna* for reproduction, TWA and pulse, Eschelisbach

Start Date 03.03.2015 End Date 30.08.2015

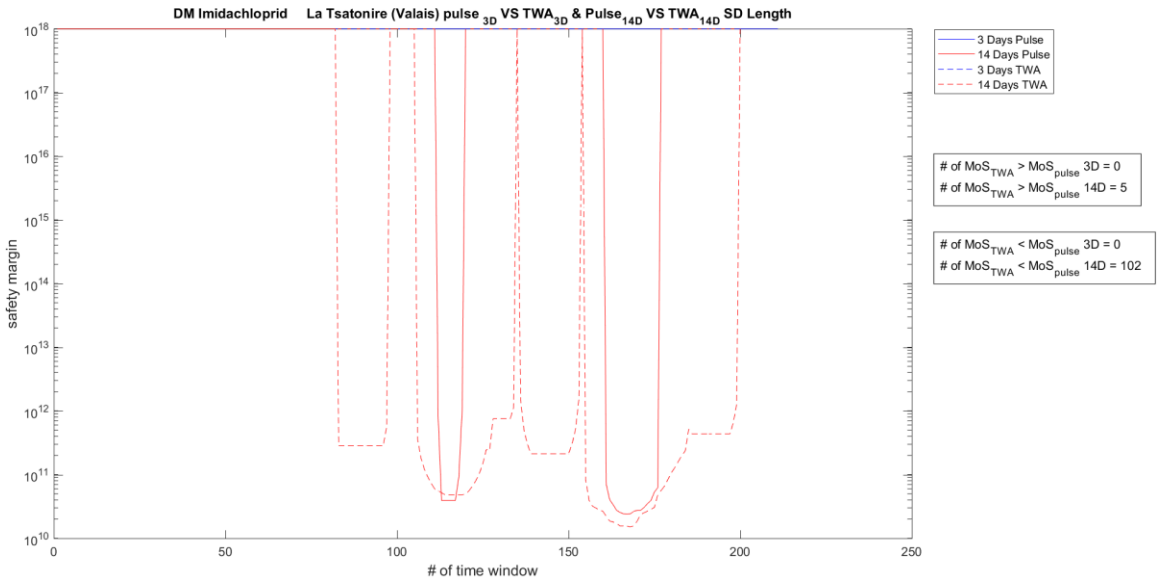

Figure 10. Margins of Safety of *Daphnia magna* for length, TWA and pulse, La Tsatonire

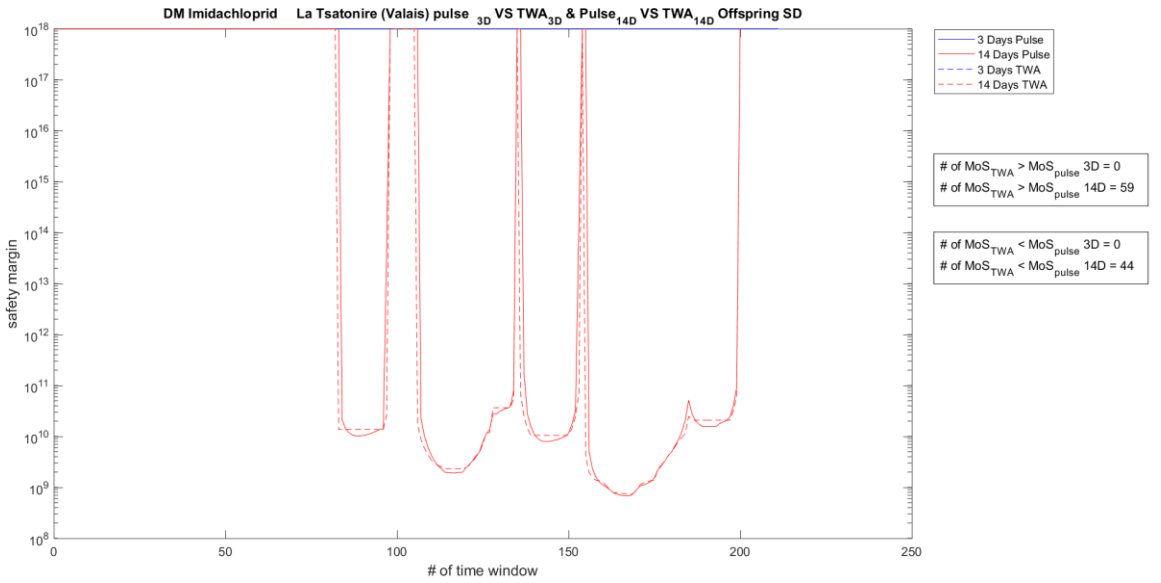

Figure 11. Margins of Safety of *Daphnia magna* for reproduction, TWA and pulse, La Tsatonire

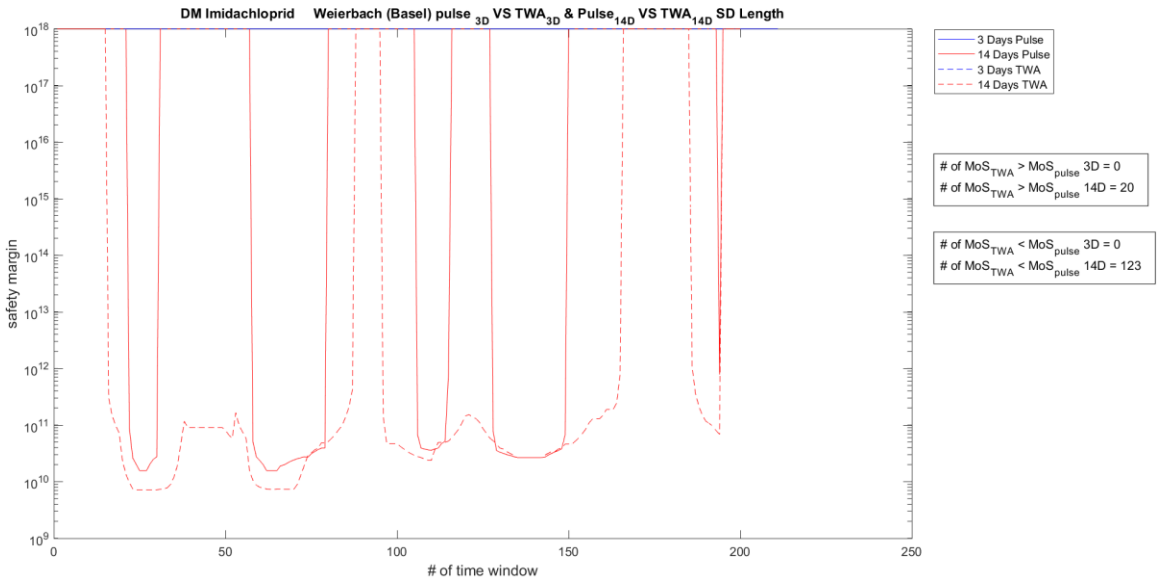

Figure 12. Margins of Safety of *Daphnia magna* for length, TWA and pulse, Weierbach

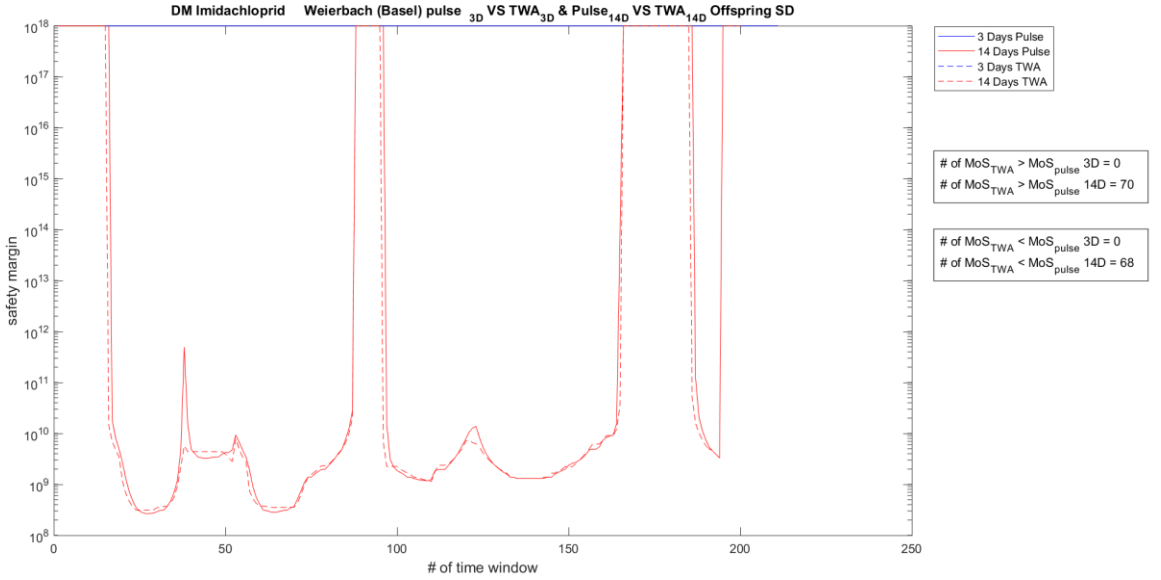

Figure 13. Margins of Safety of *Daphnia magna* for reproduction, TWA and pulse, Weierbach

Final Report

Simulation based assessment of Haber's law using modelling approaches (TKTD, GUTS)

- Algae and Lemna

Author: **Roland Kuhl**
Dr. Elke Zimmer

Study Completion Date: August 18, 2017

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1. Survey of the Study

1.1 General Information

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Ibacon Study No.: 120661253

Project Staff:

Test Facility Management: Dr. Melanie Lichtenberger

Study Director: Roland Kuhl

1.2 Archiving

The following documents and materials will be archived for the at least 3 years:

- All electronic raw data
- the electronic final report
- any electronic final report amendment or any revised final report

Following the date on which the final report at

ibacon GmbH
Germany

After the archiving period, all raw data or material relating to the study will be discarded without the Sponsor's prior written consent.

1.3 Final Report Approval

Dr. Elke Zimmer
für

Study Director: Roland Kuhl

i. A. E. Zimmer

date: 31.08.2017

Test Facility Management: Dr. Melanie Lichtenberger

date: August 31, 2017

2. Materials and Methods

2.1 Definitions

Margin of Safety: The margin of safety is the value witch which a concentration is multiplied to lead to inhibition of growth in the simulated population compared to a control population.

Time window: A 3 day or 14 day timespan for which the time weighted average of all measured concentrations is calculated. Since two samplings were performed each day, two timeframes were used for each day.

209 time windows were calculated for the 3d TWA simulations and 198 time windows were calculated for the 14d TWA simulations.

Underestimation: A margin of safety is considered as underestimation if the calculated margin of safety for a specific start day of the time window is calculated to be lower (when using TWA) than the respective margin of safety from the real exposure profile.

2.2 Simulations for *Algae and Lemna*

Test Items: Diuron, Metazachlor

Test Species: *Lemna gibba, Lemna minor, Scenedesmus subspicatus, Synechococcus* sp.,

Software: Matlab 2017a 64-bit version 9.2.0.556.344 (March 27, 2017)

Simulations: The TWA and pulse concentration values of 3 and 14 day time windows were calculated from the exposure profiles and used to calculate growth rate and growth values via a regression decision tree model based on the growth rate test results.

- First, a cubic interpolation was performed on the growth rate test results to obtain a detailed dose-response curve.
- Subsequently, a regression decision tree model was developed based on the growth rate test results. This model was used to calculate the growth rate for the concentration of each time point in the TWA and pulse time windows.
- The growth values at the end of each time window were the calculated via the function for growth rate given in the raw data file ($N(t)=N(0)*\exp(r*t)$). Simulations on the growth were performed for the 3d and 14d TWA and pulse time window by considering the starting value for growth is 1.
- The margin of safety was calculated to obtain 50% of the growth at the end of each time window in comparison to the control growth.

As a maximum safety margin, we assumed $1x10^4$. As no effect was observed for Lemna minor in the Eschelisbach, the simulation was repeated with a maximum safety margin of $1x10^9$.

3. Results for *Algae and Lemna*

3.1 Simulation results

Overall, MoS for the TWA simulations is very close to the real exposure simulations. As expected, the 14d TWA simulations generally resulted in lower MoS than the 3d TWA simulations.

The graphs show the safety margin as $1x10^9$ when either the concentration was zero or when the concentration times the safety factor was still lower than the no-effect concentration.

Table 1. The 3 most toxic time windows leading to the lowest margins of safety for 3d simulations

	Lowest Margin of Safety											
Chemical	3d TWA						3d pulse					
and Location	1st	d	2nd	d	3rd	d	1st	d	2nd	d	3rd	d
Metazachlor	***Lemna gibba***											
Bern	885	1	885	1	885	2	885	1	885	1	885	2
Ticino	1402	20	1651	20	1969	19	1402	20	1969	20	2500	19
Weierbach	50.0	50	50.0	50	62.9	49	50.0	50	56.5	50	80.5	51
Diuron	***Lemna minor***											
Eschelisbach	17399	31	17399	32	21836	68	22963	9	32130	9	32130	10
La Tsatonire	30.2	38	30.2	39	30.2	39	30.2	38	30.2	39	30.2	39
Weierbach	1124	58	1124	59	1124	59	10000	1	10000	1	10000	2
Metazachlor	***Scenedesmus subspicatus***											
Bern	119	1	119	1	119	2	119	1	119	1	119	2
Ticino	188	20	224	20	272	19	188	20	224	20	266	19
Weierbach	7.26	50	8.23	50	10.1	49	7.01	50	8.23	50	9.39	49
Diuron	***Synechococcus* sp.**											
Eschelisbach	50.0	31	50.0	32	51.8	68	54.5	9	54.5	9	195	31
La Tsatonire	0.074	38	0.074	39	0.074	39	0.074	38	0.074	39	0.074	39
Weierbach	2.65	58	2.65	59	2.65	59	11.6	58	11.6	59	11.6	59

Table 2. The 3 most toxic time windows leading to the lowest margins of safety for 14d simulations

Chemical and Location	Lowest Margin of Safety 14d TWA 1st	d	2nd	d	3rd	d	14d pulse 1st	d	2nd	d	3rd	d
Metazachlor							***Lemna gibba***					
Bern	461	27	461	28	461	28	461	1	461	27	461	27
Ticino	1374	15	1374	15	1374	16	1041	15	1041	15	1041	16
Weierbach	28.4	50	28.7	49	30.2	49	26.1	50	26.5	49	27.3	49
Diuron							***Lemna minor***					
Eschelisbach	17819	4	17819	5	19589	4	13933	4	13933	5	14936	4
La Tsatonire	13.9	35	13.9	36	13.9	37	13.1	35	13.1	36	13.1	37
Weierbach	1454	53	1454	53	1454	54	10000	1	10000	1	10000	2
Metazachlor							***Scenedesmus subspicatus***					
60.8	60.8	27	60.8	28	60.8	28	62.9	1	73.6	27	73.8	27
182	182	15	182	15	182	16	138	15	138	15	138	16
3.78	3.78	49	3.78	50	3.97	49	4.53	49	4.53	50	4.59	48
Diuron							***Synechococcus* sp.**					
32.0	32.0	4	32.0	5	34.9	4	32.4	4	32.4	5	37.4	4
0.027	0.027	35	0.027	36	0.027	37	0.026	35	0.026	36	0.026	37
2.23	2.23	53	2.23	53	2.23	54	11.6	53	11.6	53	11.6	54

Table 3. Comparison of MoS of simulation results of Lemna and Algae from 3d and 14d TWA calculations with respective pulse exposure results

Chemical and Location	Comparison of TWA simluation vs. real exposure scenario							
	3d TWA < pulse		3d TWA > pulse		14d TWA < pulse		14d TWA > pulse	
Metazachlor				***Lemna gibba***				
Bern	26	12 %	0	0 %	21	11 %	80	40 %
Ticino	22	11 %	0	0 %	28	14 %	21	11 %
Weierbach	78	37 %	0	0 %	4	2 %	91	46 %
Diuron				***Lemna minor***				
Eschelisbach	28	13 %	0	0 %	67	34 %	15	8 %
La Tsatonire	98	47 %	0	0 %	67	34 %	131	66 %
Weierbach	7	3 %	0	0 %	16	8 %	0	0 %
Metazachlor				***Scenedesmus subspicatus***				
Bern	7	3 %	44	21 %	50	25 %	98	49 %
Ticino	4	2 %	36	17 %	6	3 %	60	30 %
Weierbach	8	4 %	56	27 %	105	53 %	13	7 %
Diuron				***Synechococcus* sp.**				
Eschelisbach	28	13 %	0	0 %	82	41 %	0	0 %
La Tsatonire	93	44 %	7	3 %	160	81 %	8	4 %
Weierbach	8	4 %	0	0 %	19	10 %	0	0 %

4. References

Jager T., Albert C., Preuss T.G., Ashauer R. (2011). General unified threshold model of survival - a toxicokinetic-toxicodynamic framework for ecotoxicology. Environmental Science & Technology 45 (7): 2529–2540. DOI: 10.1021/es103092a

5. Distribution of the Final Report

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Appendix I: Tables and Figures

Plots for *Lemna gibba* and Metazachlor

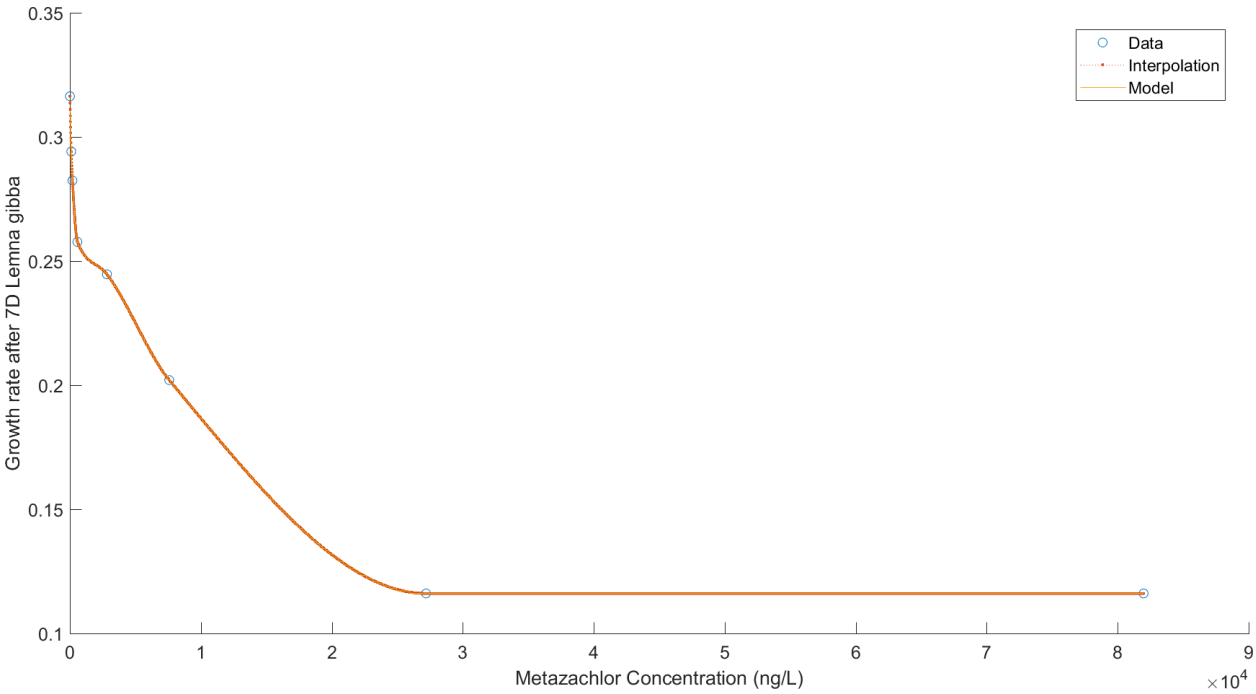

Figure 1. Interpolation of toxicity of *Lemna gibba* to Metazachlor

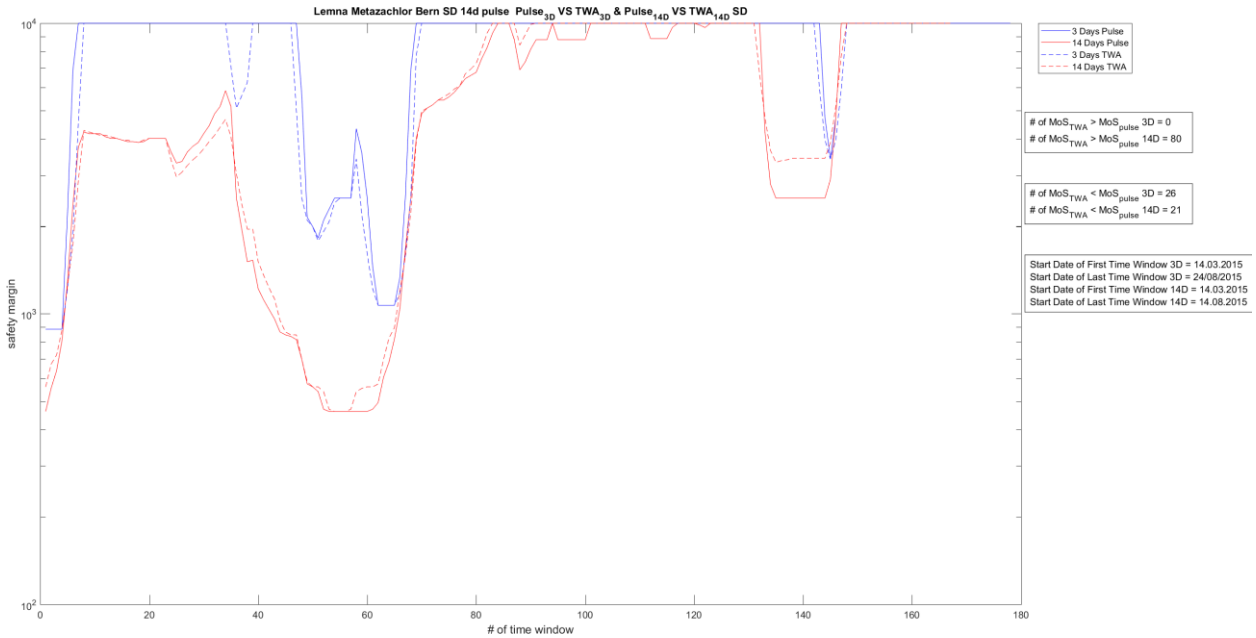

Figure 2. Margins of Safety for Bern with 3d pulse, 3 day TWA, 14d pulse and 14 day TWA

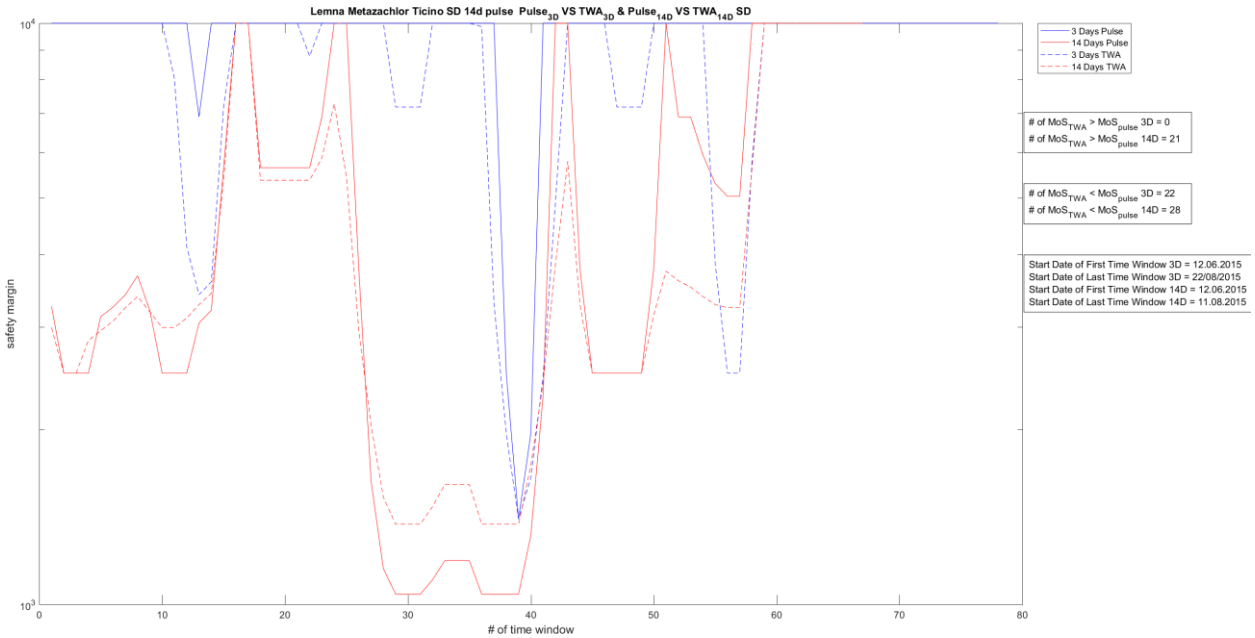

Figure 3. Margins of Safety for Ticino with 3d pulse, 3 day TWA, 14d pulse and 14 day TWA

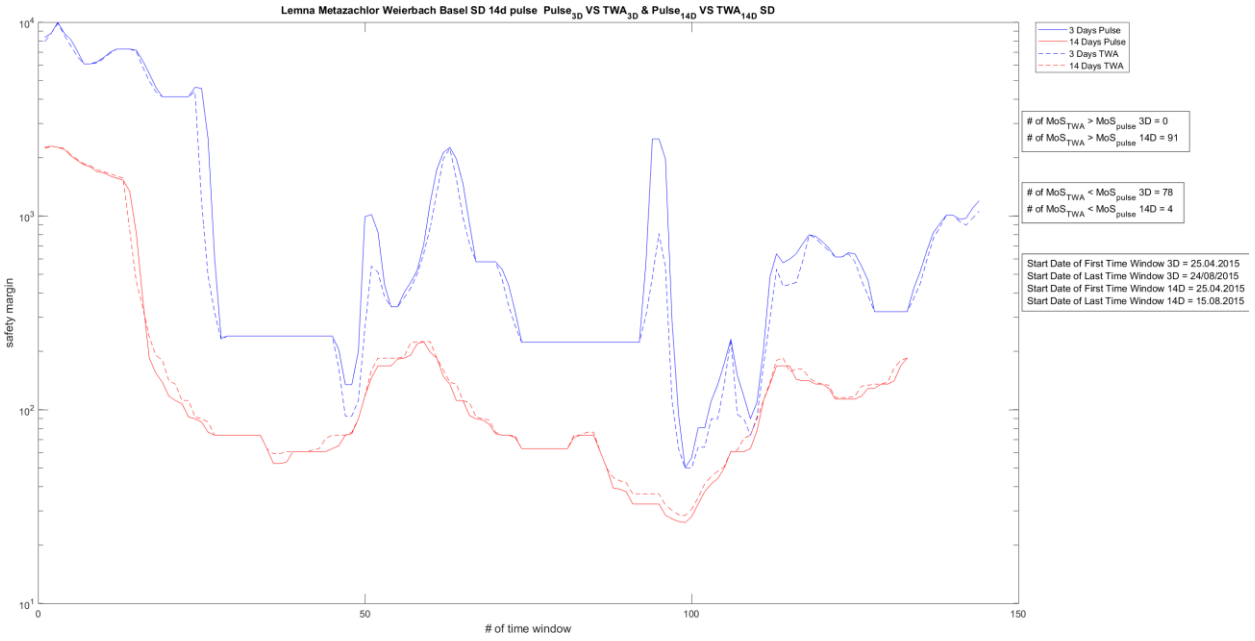

Figure 4. Margins of Safety for Weierbach with 3d pulse, 3 day TWA, 14d pulse and 14 day TWA

Plots for *Lemna minor* and Diuron

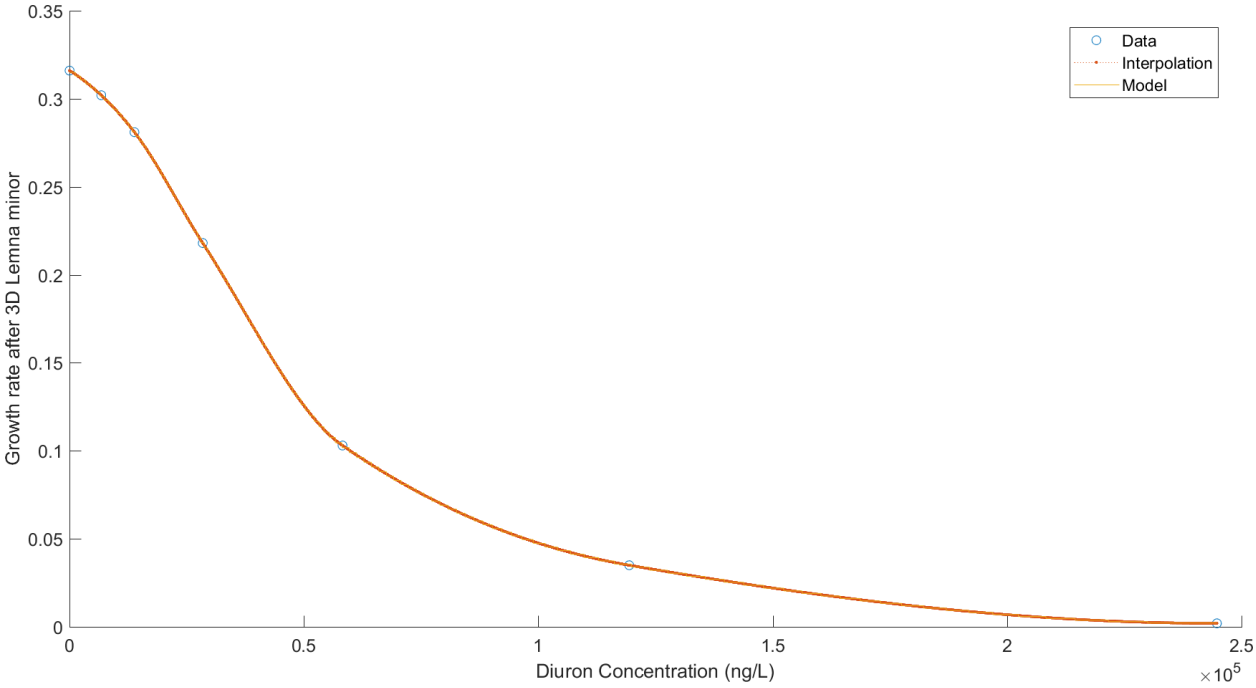

Figure 5. Interpolation of toxicity of *Lemna minor* to Diuron

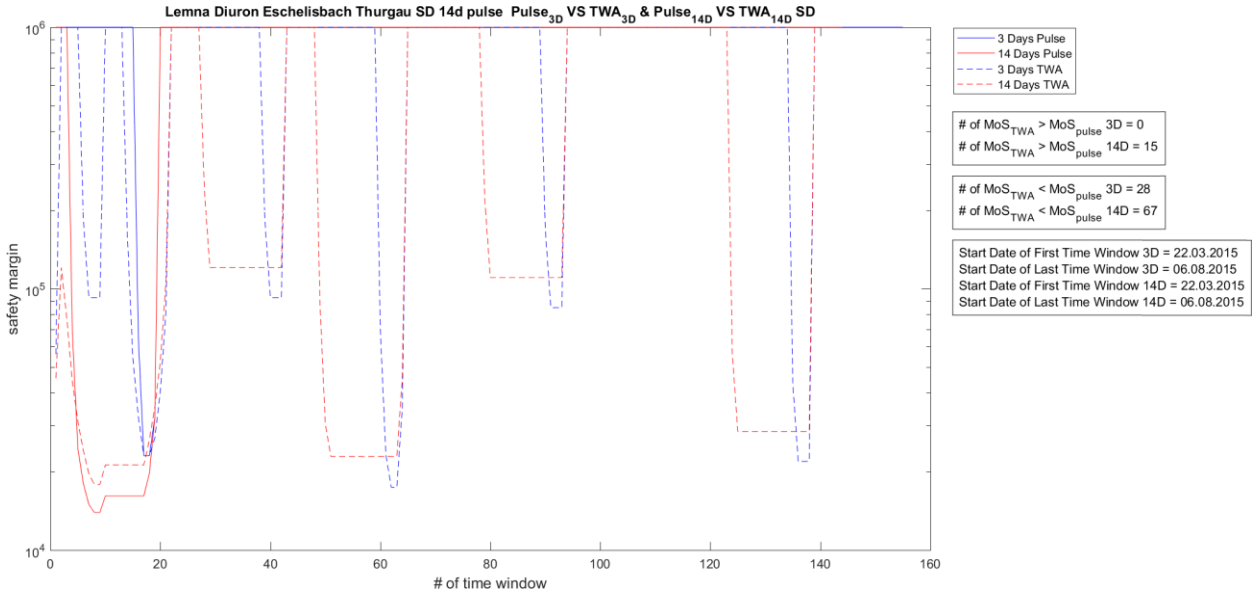

Figure 6. Margins of Safety for Eschelisbach with 3d pulse, 3 day TWA, 14d pulse and 14 day TWA

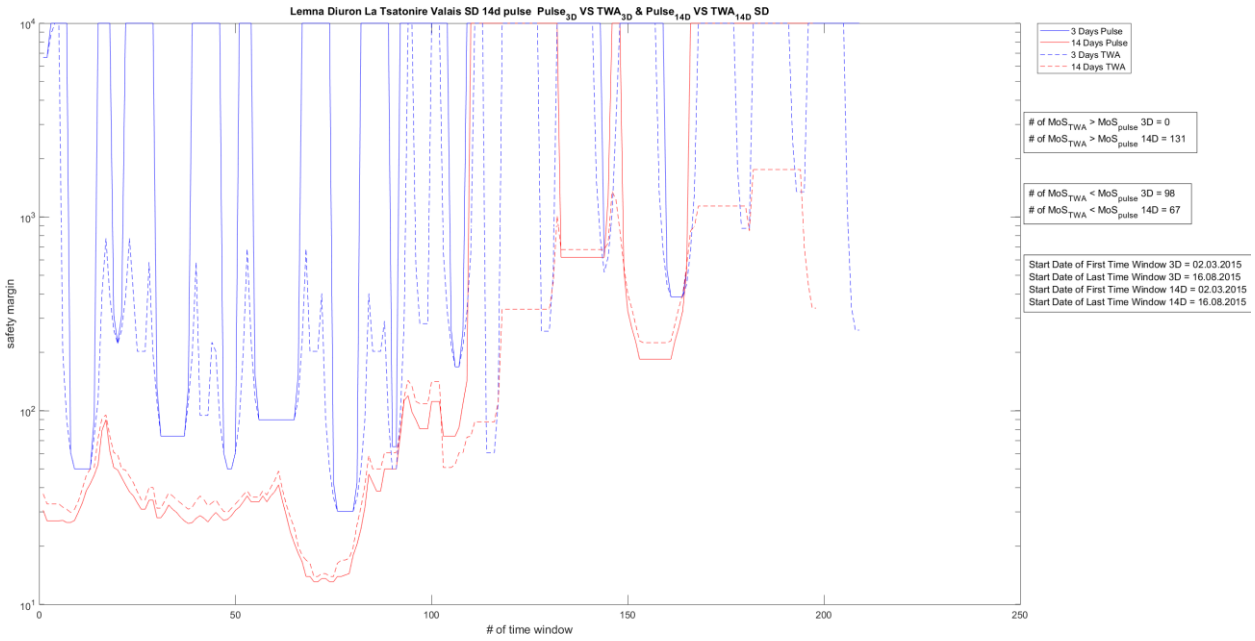

Figure 7. Margins of Safety for La Tsatonire with 3d pulse, 3 day TWA, 14d pulse and 14 day TWA

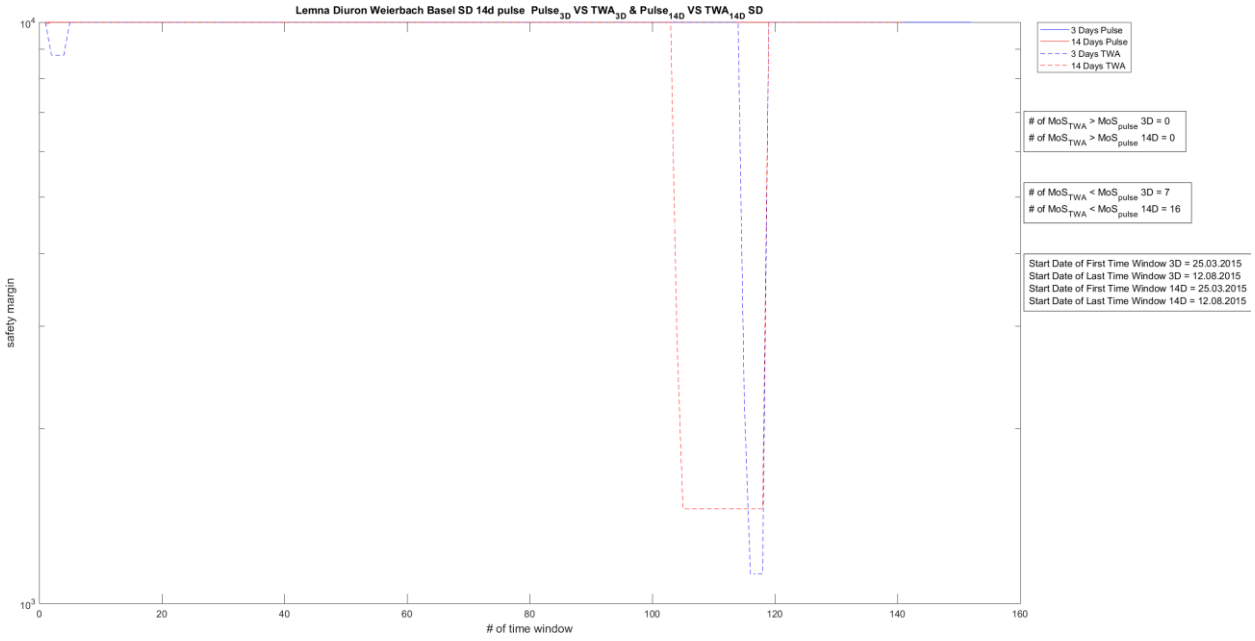

Figure 8. Margins of Safety for Weierbach with 3d pulse, 3 day TWA, 14d pulse and 14 day TWA

Plots for *Scenedesmus subspicatus* and Metazachlor

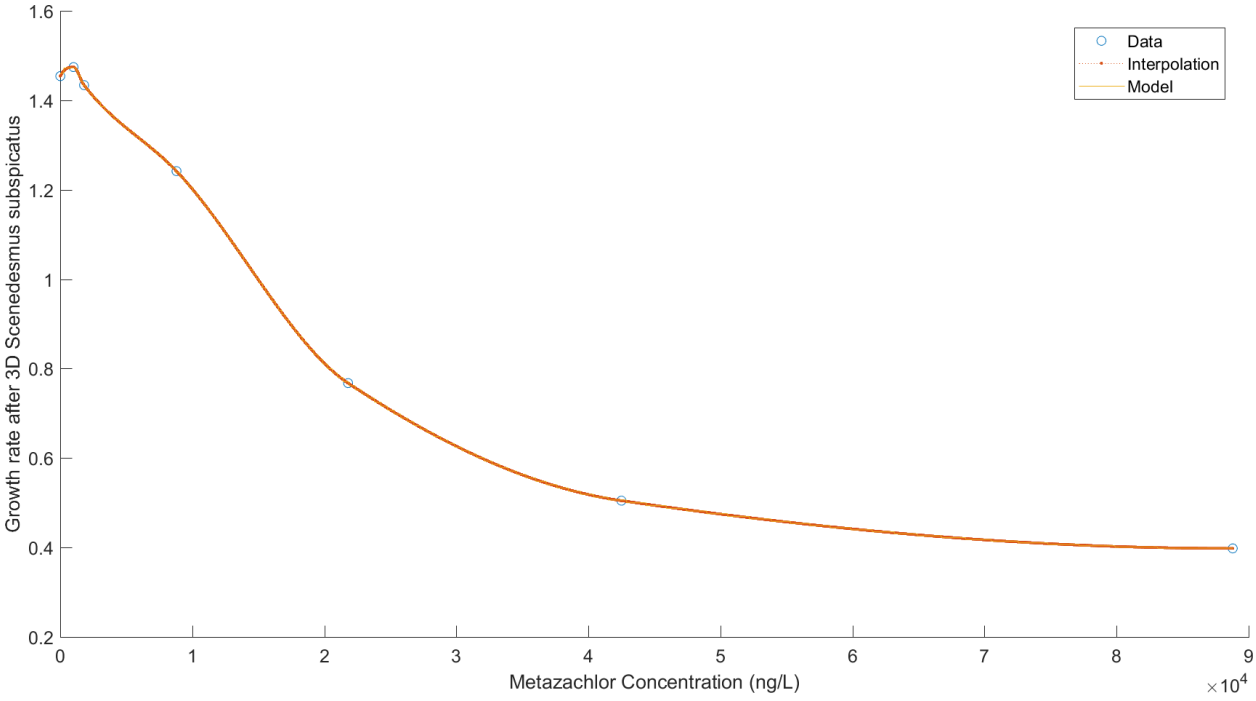

Figure 9. Interpolation of toxicity of *Scenedesmus subspicatus* to Metazachlor

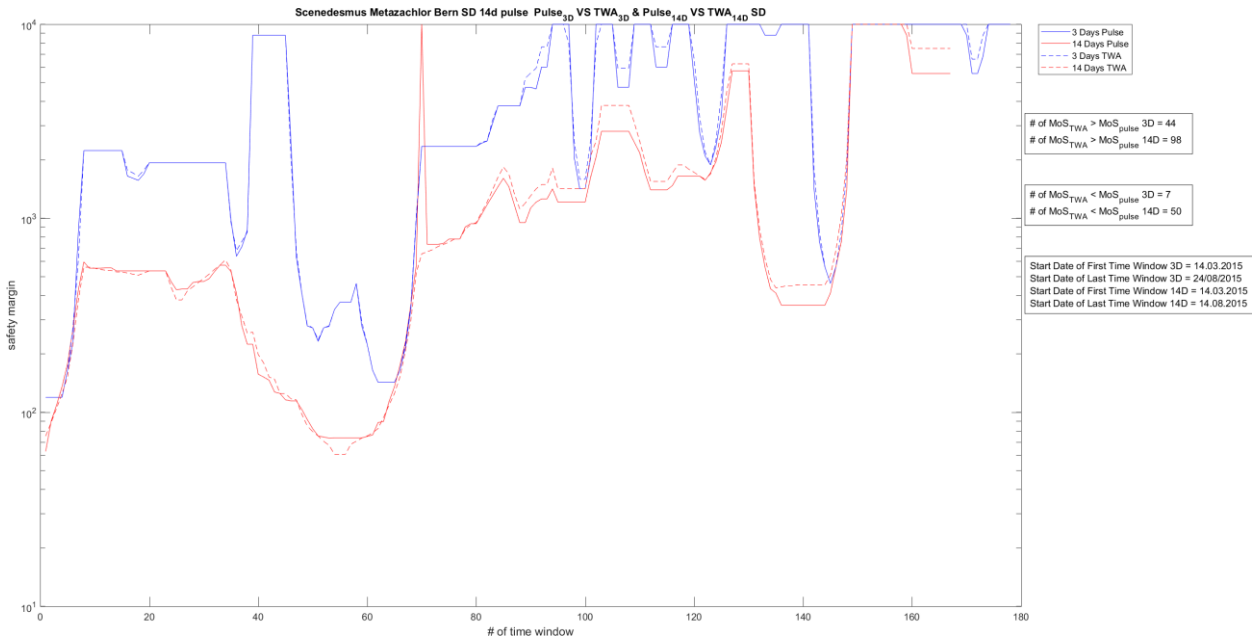

Figure 10. Margins of Safety for Bern with 3d pulse, 3 day TWA, 14d pulse and 14 day TWA

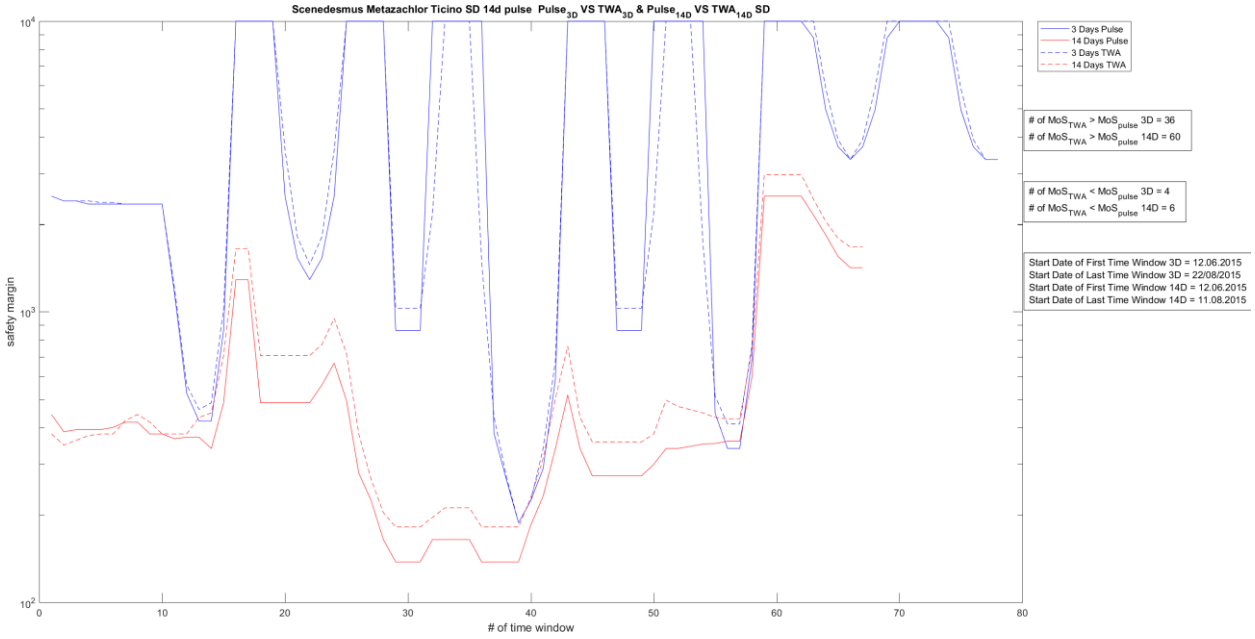

Figure 11. Margins of Safety for Ticino with 3d pulse, 3 day TWA, 14d pulse and 14 day TWA

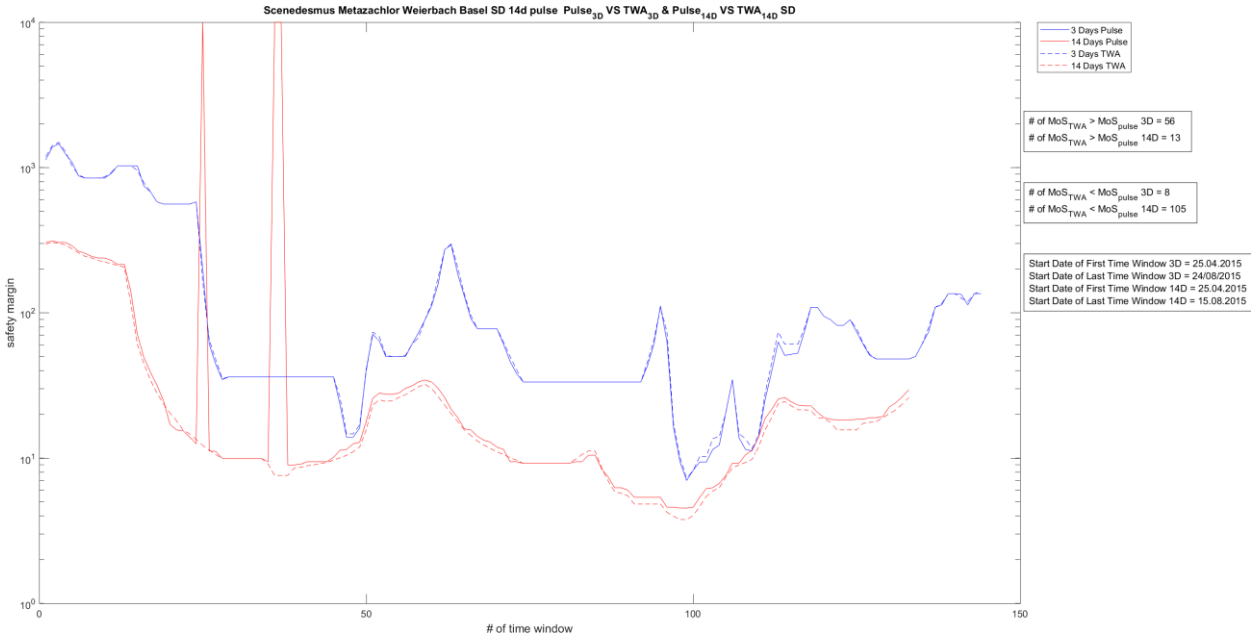

Figure 12. Margins of Safety for Weierbach with 3d pulse, 3 day TWA, 14d pulse and 14 day TWA

Plots for *Synechococcus* sp. and Diuron

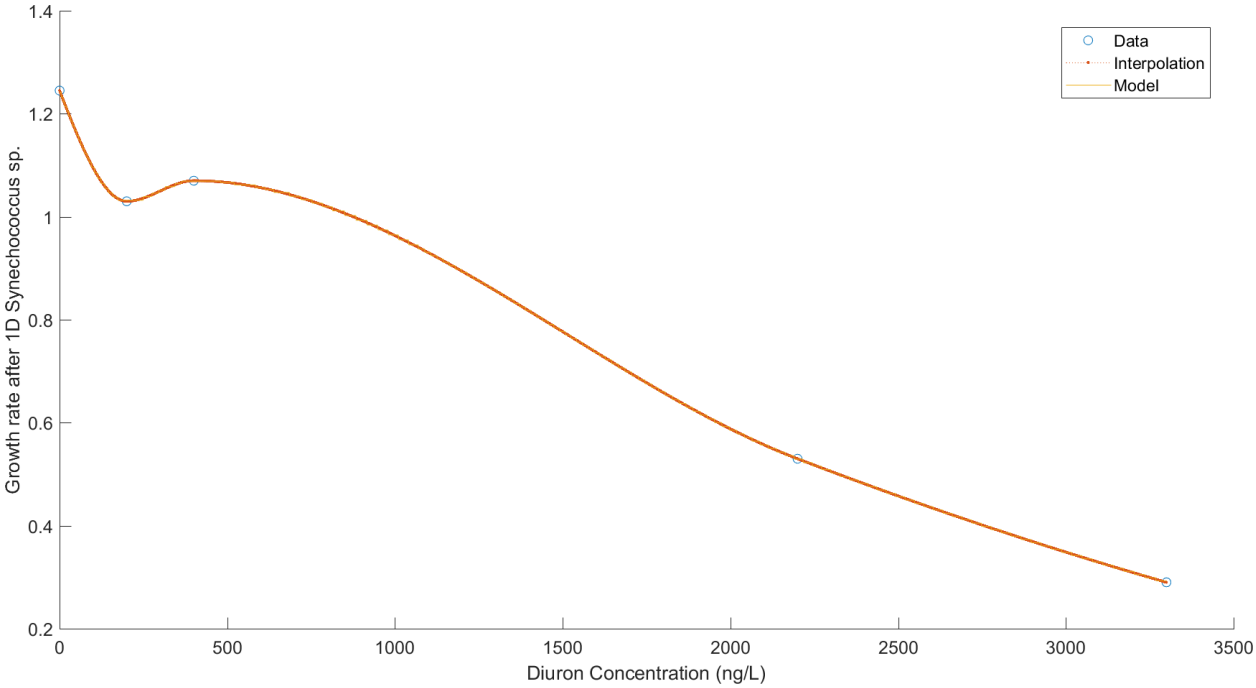

Figure 13. Interpolation of toxicity of *Synechococcus* sp. to Metazachlor

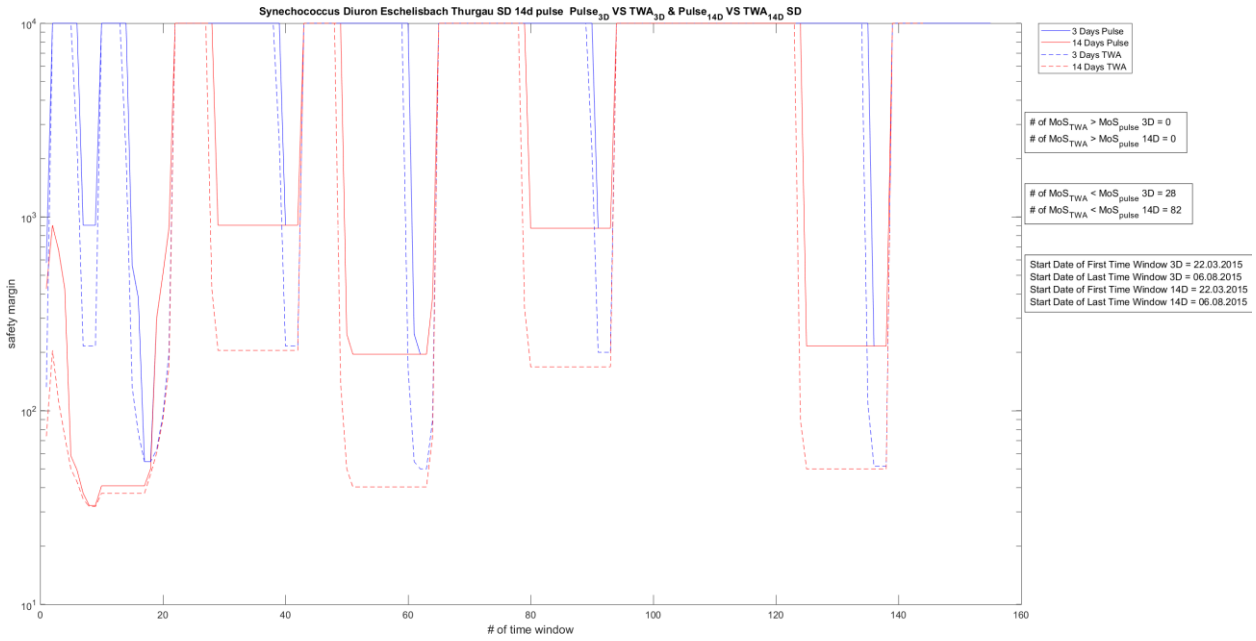

Figure 14. Margins of Safety for Eschelisbach with 3d pulse, 3 day TWA, 14d pulse and 14 day TWA

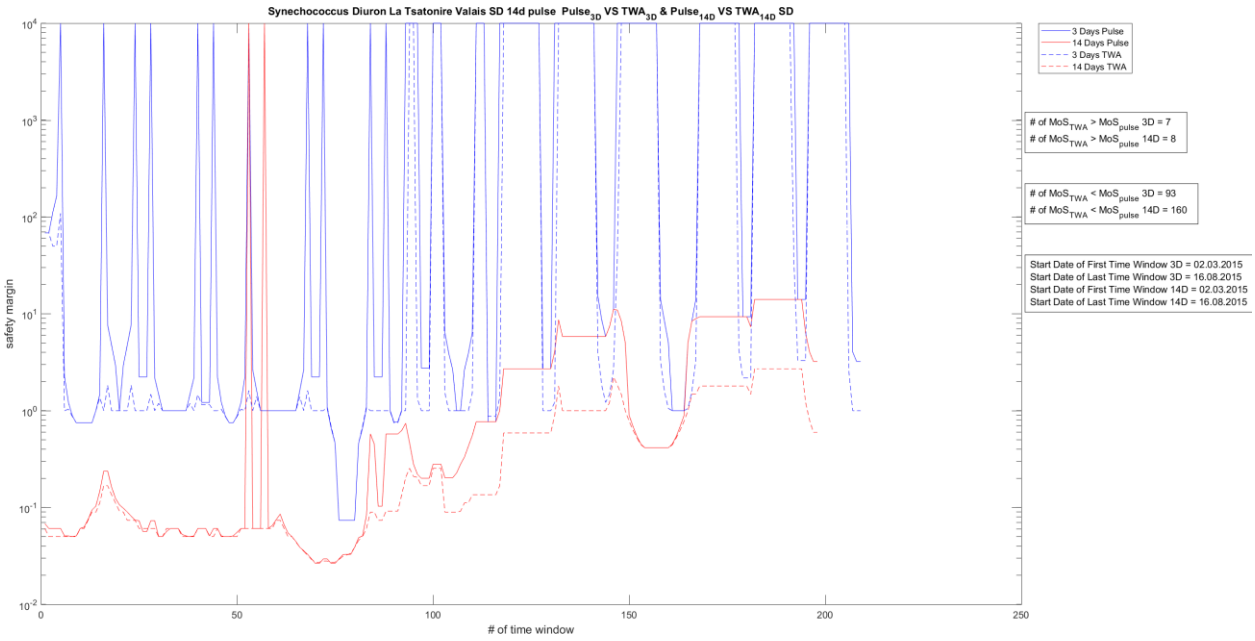

Figure 15. Margins of Safety for La Tsatonire with 3d pulse, 3 day TWA, 14d pulse and 14 day TWA

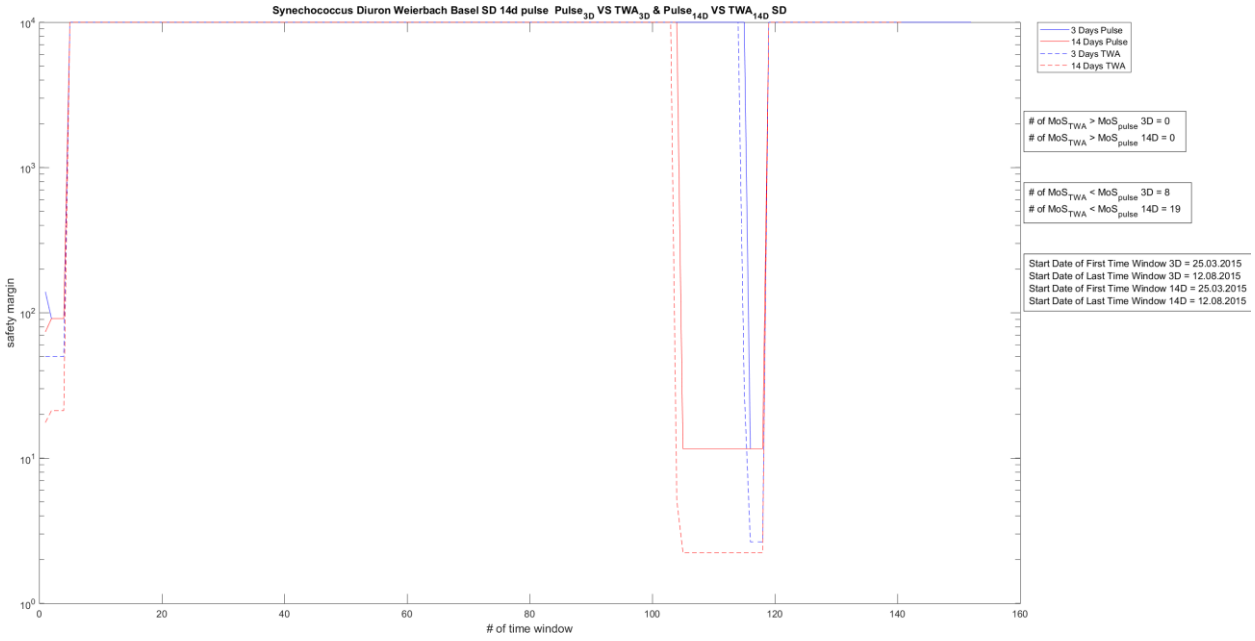

Figure 16. Margins of Safety for Weierbach with 3d pulse, 3 day TWA, 14d pulse and 14 day TWA