OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION
WASHINGTON, D.C. 20460

MEMORANDUM

DATE: January 22, 2024

SUBJECT: Evaluation of the Developmental Neurotoxicity Potential of Malathion/Malaoxon to Inform the FQPA Safety Factor

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The conclusions conveyed in this assessment were developed in full compliance with *EPA Scientific Integrity Policy for Transparent and Objective Science*, and EPA Scientific Integrity Program's *Approaches for Expressing and Resolving Differing Scientific Opinions.* The full text of *EPA Scientific Integrity Policy for Transparent and Objective Science*, as updated and approved by the Scientific Integrity Committee and EPA Science Advisor can be found here: https://www.epa.gov/sites/default/files/2014-02/documents/scientific_integrity_policy_2012.pdf. The full text of the EPA Scientific Integrity Program's *Approaches for Expressing and Resolving Differing Scientific Opinions* can be found here: https://www.epa.gov/scientific-integrity/approaches-expressing-and-resolving-differing-scientific-opinions.

This memorandum serves as the supporting document for the second revised human health draft risk assessment of the dietary, occupational, non-occupational, and aggregate exposure from registered uses of malathion (W. Britton, Task Group No. 00491975, 22-JAN-2024) and supersedes the previous memo (A. Britt, TXR 0058560, D467211, 10-APR-2023).

Introduction

The organophosphates (OPs) are a class of pesticides that have an established mode of action/adverse outcome pathway (MOA/AOP) involving inhibition of the enzyme acetylcholinesterase (AChE) via phosphorylation of the serine residue at the active site of the enzyme. This inhibition leads to accumulation of acetylcholine (a neurotransmitter) and ultimately to neurotoxicity in the central and/or peripheral nervous system. The need for additional developmental neurotoxicity (DNT) data to assess potential effects in the developing young, including the DNT guideline study for any pesticide, is considered in the context of the entire database and in accordance with the 40 CFR Part 158 toxicology data requirements for pesticides[1]. Based on the available data for OPs, EPA previously issued data call-ins for *in vivo* DNT studies; however, none of the 18 submitted DNT studies for OPs identified endpoints that were more sensitive than AChE inhibition. Consequently, EPA has continued to use AChE inhibition as the critical endpoint for OP human health risk assessments. However, in recent years, scientific evidence has raised uncertainty about whether AChE inhibition will be protective of potential neurodevelopmental effects for OPs, especially given the lack of a MOA/AOP for the potential neurodevelopmental effects.

In 2015, EPA released a literature review on neurodevelopmental effects and Food Quality Protection Act Safety Factor (FQPA SF) determination for the OP pesticides (A. Lowit, D331251, 15-SEP-2015). The review was then updated in 2016 to incorporate additional studies and address public comments (A. Aldridge et al., D437043, 29-DEC-2016). In the 2015/2016 review, data from three primary lines of evidence – epidemiological studies, studies in laboratory animals, and *in vitro* assays – were evaluated in a weight of evidence (WOE) approach to assess the DNT potential of OPs. Although the MOA/AOP is not established for any potential developmental neurotoxic outcomes, OPP took a conservative approach by performing the 2015/2016 review for the OPs as a group based on the assumption that, like AChE inhibition and subsequent neurotoxicity, DNT outcomes would share a common MOA/AOP.

At the time of the 2015/2016 review, uncertainties regarding potential neurodevelopmental effects and their relative sensitivity to AChE inhibition for OPs was most notably raised by epidemiological studies; however, limitations were identified in these studies that make it difficult to causally or quantitatively link exposure of individual OPs to the investigated outcomes. Ultimately, out of an abundance of caution, the 10X FQPA SF for the OPs was retained at that time due to the overarching uncertainties in the human dose-response relationship for potential neurodevelopmental outcomes and its quantitative relationship to AChE inhibition. As a result, in the last risk assessment for malathion and its metabolite/degradate malaoxon (S. Shalu, D414107, 09-JUN-2016), the FQPA 10X SF was retained or a database uncertainty factor was applied for the population subgroups that include infants, children, youths, and women of childbearing age for all exposure scenarios.

Recognizing the uncertainty in the human dose-response relationship for neurodevelopmental outcomes, EPA has pursued the development of approaches to facilitate quantitative or semi-quantitative comparisons between doses which elicit AChE inhibition and those which are associated with potential neurodevelopmental outcomes. Since the 2015/2016 review, high quality data on underlying biological processes of neurodevelopment have become available as a result of an international effort to develop new approach methodologies (NAMs) for evaluating DNT hazard. This

[1] https://www.ecfr.gov/current/title-40/chapter-I/subchapter-E/part-158?toc=1

international effort recognized the strengths and limitations of the available DNT studies through a series of meetings with scientists, regulators, and stakeholders (Lein et al., 2007; Coecke et al., 2007; Crofton et al., 2011; Bal-Price et al., 2012; Aschner et al., 2017; Fritsche et al., 2017; Fritsche et al., 2018a; Fritsche et al., 2018b; Bal-Price et al., 2018; Sachana et al., 2019), leading to the development of a battery of *in vitro* assays that assess processes critical to development of the nervous system (referred to hereafter as DNT NAM battery), and provide chemical-specific evaluation of DNT hazard potential. By focusing on critical biological processes underlying neurodevelopment, the DNT NAM battery can provide relevant information regarding DNT hazard potential of individual chemicals and evaluate early perturbations that are difficult to obtain or evaluate *in vivo*. Therefore, assessment of whether a chemical may impact these upstream critical processes provides an evaluation of its ability to yield a myriad of potential downstream DNT impacts, including complex neurological deficits seen in the human population ranging from subtle learning disabilities to severe neural tube defects (*e.g.,* spina bifida).

The *in vitro* data from the DNT NAM battery can provide a scientifically robust, data-driven basis for evaluating potential DNT hazard and its quantitative relationship to AChE inhibition for individual OPs. In 2020, EPA convened a Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) to review the DNT NAM battery with the OPs as a case study[2]. Overall, the SAP agreed that the current DNT NAM battery reflects, if not directly models, critical processes for neurodevelopment and that data from the battery can be used as part of a WOE evaluation. Activity observed in the DNT NAM battery can also be used in kinetic models, such as high-throughput toxicokinetic (HTTK) or refined physiologically-based pharmacokinetic (PBPK) models, where the *in vitro* concentrations that are associated with bioactivity observed in the DNT NAM battery can be directly compared with an internal dose metric (e.g., average blood concentration) associated with chemical-specific points of departure (PODs). For OPs, such comparison evaluates the relative sensitivity of activity in the DNT NAM battery to AChE inhibition given the PODs for OP human health risk assessments are based on 10% AChE inhibition. AChE endpoints can be derived from *in vivo* animal studies or predicted using PBPK models when available. Therefore, *in vitro* data from the DNT NAM battery can be used as part of a WOE approach, to characterize the uncertainty in the dose-response relationship for neurodevelopmental outcomes and AChE inhibition by comparing the average blood concentration associated with a 10% inhibition of AChE (from *in vivo* animal studies) with the *in vitro* bioactive concentrations from the DNT NAM battery assays.

Notably, the data from the DNT NAM battery for numerous OP compounds has demonstrated that differences exist across OP chemicals with respect to their potential to elicit neurodevelopmental effects. No consistent pattern (e.g., differences in degree of activity/inactivity; activity in different assays that represent different critical processes) has emerged to suggest that all OPs share a common pathway for potential DNT or to support the assumption that all OP compounds have similar concerns related to DNT. The differences observed in the DNT NAM battery data also emphasize differences in DNT potential across OPs that were previously identified in epidemiological and laboratory animal studies. Accordingly, EPA has reevaluated its approach to assessing the DNT potential of the OPs.

Based on the best available science, OPP has determined that DNT potential of OPs should be evaluated on a chemical-by-chemical basis, not as a group (M. Perron, TXR 0058584, D467385, 10-APR-

[2] https://www.regulations.gov/document/EPA-HQ-OPP-2020-0263-0006

2023). Moving forward, as with all pesticides subject to FQPA, EPA will continue to assume a default 10X safety factor to protect for infants and children unless reliable data exists to support application of a different safety factor. An appropriate FQPA safety factor for each OP will be assessed on an individual chemical basis taking into consideration the strengths and limitations of all scientifically sound data and information. To inform the FQPA SF determination for OPs, DNT potential will be evaluated using chemical-specific data for each OP in a WOE evaluation. This WOE evaluation considers whether the OP compound has the potential to elicit DNT outcomes, as well as the relative sensitivity of potential DNT to AChE inhibition (i.e., comparison of relevant dose(s) where potential DNT outcomes may occur in relation to AChE inhibition).

Since malathion must be metabolized (activated) to its oxon (malaoxon) to inhibit AChE, this document provides an evaluation of chemical-specific data for both malathion and malaoxon. The following sections will provide:

- an overview of the DNT NAM battery and methods used to analyze the data from the battery;
- a summary of the results from the DNT NAM battery for malathion and malaoxon;
- a comparison of the *in vitro* concentrations from the DNT NAM battery to the average blood concentrations associated with PODs for 10% AChE inhibition
- a summary of available *in vivo* DNT studies for malathion in laboratory animals;
- a summary of epidemiological studies that investigated associations between malathion and DNT outcomes;
- discussion of the results from all lines of evidence, including the strengths and limitations of each; and
- EPA's conclusion on the DNT potential for malathion/malaoxon, including the quantitative relationship of potential DNT outcomes to AChE inhibition, to inform the FQPA SF.

Overview of the DNT NAM battery

The current DNT NAM battery consists of multiple *in vitro* assays that utilize either human or rat neural cell models. Assays in the DNT NAM battery were developed by the US EPA Office of Research and Development (ORD) and international collaborators, with a goal of facilitating faster, less expensive, and more human relevant DNT screening and evaluation. The overarching goal was to develop a battery of assays that measure critical neurodevelopmental processes *in vitro* including proliferation of neuroprogenitor cells, differentiation of neuroprogenitors into glial and neuronal subtypes, apoptosis, migration of neurons and oligodendrocytes, neurite outgrowth (NOG), synaptogenesis, and neural network formation (Figure 1). When development of the DNT NAM battery was initiated, it was recognized that brain development is complex, comprising of distinct neurodevelopmental processes occurring at different ages, across specific brain regions, involving many different cell types. The concept of evaluating 'key' neurodevelopmental processes was designed to address this issue, given that these processes must occur for proper nervous system development and function, and the mechanisms underlying these processes are well conserved. By focusing on critical biological processes that are the underpinnings of the apical endpoints, the DNT NAM battery can provide relevant information regarding DNT potential of individual chemicals related to critical processes of neurodevelopment and evaluate early perturbations that are difficult to obtain or evaluate *in vivo*.

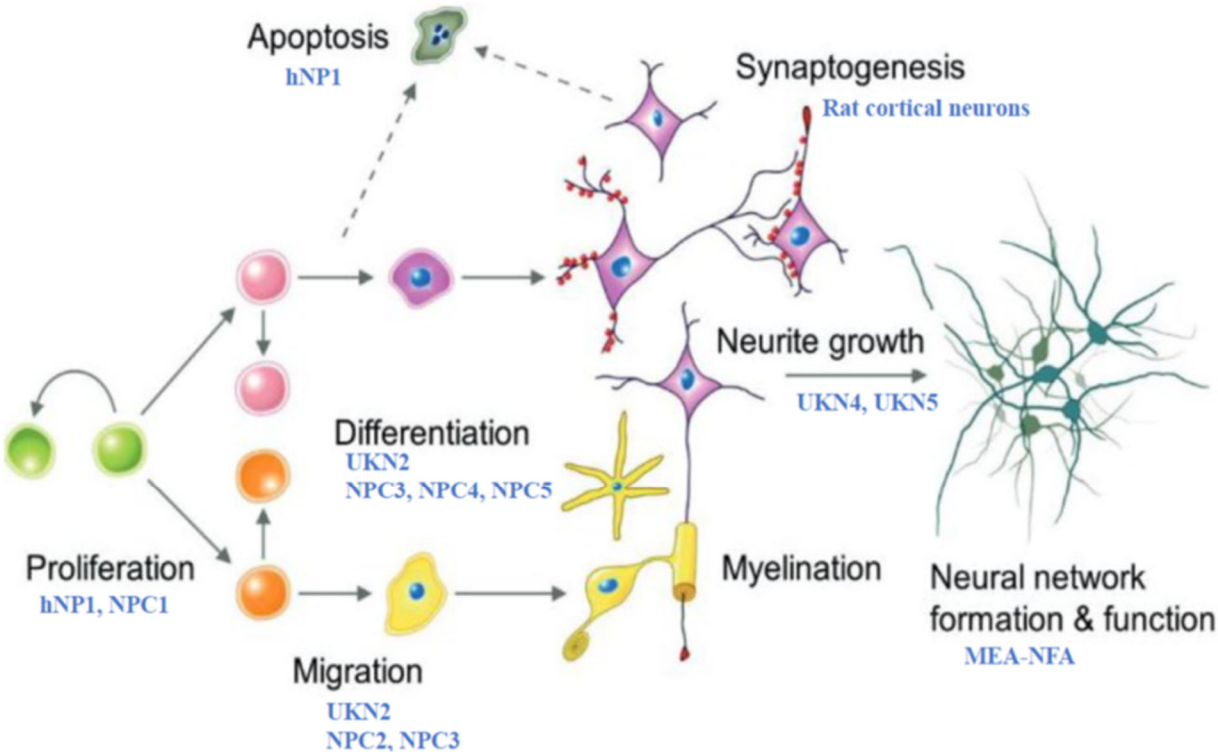
Chemical-induced alterations in these processes would indicate a potential hazard for neurodevelopment as these processes are highly conserved in mammals. Activity in these assays should not be construed as evidence of DNT *in vivo*. Although activity may be observed in the battery, it may not necessarily represent an adverse change that is typically linked to tissue-level or apical effects in a MOA/AOP. As described in the "*Toxicity Testing in the 21st Century*" report[3], to develop an AOP, not only is it necessary to establish plausible relationships among the key events, but quantitative relationships also need to be established. In other words, how much of a change in one key event is needed to result in an adverse effect at the next level of biological organization? Thus, certain exposures to a chemical may impact normal physiological responses in a way that may not necessarily be adverse. Consequently, the AOP concept requires an understanding of adaptive/homeostatic capacity of biological systems and their limits, relative to concentration and duration of exposure. OPP is taking a health-protective approach at this time by assuming that observed activity (in the form of true positive results) in the battery is associated with adversity.

Rather than trying to investigate every potential element that may be involved in a cascade of events that influence neurodevelopment (e.g., neurotransmitters, hormones, gene expression, neurodevelopmental stage, etc.), the DNT NAM battery focuses on key neurodevelopmental processes and therefore takes an integrative approach. This allows for the evaluation of the interplay of the hundreds - if not thousands - of upstream molecular steps involved in neurodevelopment and the potential consequences of their perturbation in critical processes that are associated with a wide spectrum of downstream DNT outcomes. This strategy obviates the need to elucidate every molecular step in a MOA/AOP for every potential DNT outcome, which would be a resource-intensive process in terms of time, animals, and cost. This, in turn, results in faster, more biologically and human-relevant evaluations, and ultimately health-protective decision-making.

The microelectrode array-based network formation assay (MEA-NFA; rat cortical neurons) and high-content imaging (HCI) assays for proliferation (human neural progenitor cell line (hNP1)), apoptosis (hNP1 cells), NOG (human embryonic stem (hN2) or induced pluripotent stem cell derived (CDI) neurons and rat cortical neurons), and synaptogenesis (rat cortical neurons) were developed by ORD. The assays developed by ORD have been described in detail in an Agency Issue Paper provided to the 2020 SAP. International collaborators (University of Konstanz (UKN); Leibniz Institute for Environmental Medicine (IUF)) developed assays that are both overlapping and complementary to the ORD assays (Masjosthusmann et al., 2020). Using human primary neuroprogenitor cells (Lonza) in neurosphere cultures, IUF has developed assays for proliferation (NPC1), radial glial migration (NPC2), neuronal differentiation (NPC3), and oligodendrocyte differentiation (NPC5). Assays developed at the UKN include migration of human neural crest from h9 embryonic stem cells (UKN2), NOG in Lund human mesencephalic embryonic neuronal precursor (LUHMES) cells (UKN4), and NOG in human peripheral nervous system cells (immature dorsal root ganglion) cells from h9 embryonic cells (UKN5). A complete summary of the assays and strategies can be found in Sachana *et al*., 2021. Each assay has a corresponding measurement of cytotoxicity which can be used to evaluate bioactivity occurring below the threshold of cytotoxicity. Changes in assay endpoints occuring at concentrations above the threshold of cytotoxicity indicate that they are likely associated with non-specific cytotoxic effects.

[3] http://www.nap.edu/catalog.php?record_id=11970

Figure 1. Critical neurodevelopmental processes and battery of *in vitro* assays (blue text)



The assays in the DNT NAM battery have been extensively characterized and reviewed. The methods, data from positive control and reference chemical testing, and the readiness of these assays have been evaluated and published in the peer-reviewed literature (Bal-Price *et al.*, 2018; Sachana *et al.*, 2021). For example, during development of the MEA-NFA, data from positive and negative control chemicals were first published for the assay in 2016 (Brown *et al.*, 2016), and this was followed by publication of results with reference chemicals (Frank *et al.*, 2017) that demonstrated or lacked putative evidence of DNT *in vivo*[4], and then by screening of larger sets of chemicals (Shafer *et al.*, 2019). A similar approach was followed for all the other assays in the current DNT NAM battery and the primary literature for each battery is summarized in Sachana *et al.*, 2021.

In 2020, EPA convened a Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) to review the DNT NAM battery with the OPs as a case study[5]. The Agency's Issue Paper supporting the SAP review provides additional characterization of the assays developed by EPA, including information on coefficients of variation, metrics of assay performance, and intralaboratory reproducibility. Overall, the SAP agreed that the current DNT NAM battery reflects, if not directly models, critical processes for neurodevelopment and that data from the battery can be used as part of a WOE evaluation, but also noted several processes and cell types that the panel believed to be missing in the battery. As discussed in the Agency's response to the SAP[6], the current battery is not entirely lacking these processes and cell types and/or these perceived limitations could be addressed by

[4] Note: Some chemicals were tested using doses and/or exposure routes that are not relevant for human health risk assessment.

[5] https://www.regulations.gov/document/EPA-HQ-OPP-2020-0263-0006

[6] https://www.regulations.gov/document/EPA-HQ-OPP-2020-0263-0057

utilizing information from other available studies. The panel recommended the DNT battery "be a living and evolving process that can be revised and improved with new technology, assays, information on validity and reliability and *in vivo* translation"; however, the panel also noted that "this is not meant to preclude the ability of the Agency to utilize all valid and relevant data in their efforts to determine risks for human health".

International review and acceptance of the battery has also progressed since the 2020 SAP. Some organizations, such as European Food Safety Authority (EFSA) and European Chemicals Agency (ECHA), currently consider data from the DNT NAM battery as part of their evaluations. Further, an Expert Group on DNT was convened by the Organization of Economic Cooperation and Development (OECD) to develop a guidance document that describes the use of the battery as part of an Integrated Approach for Testing and Assessment (IATA) for DNT[7]. This guidance went through two rounds of review by OECD member states and partners (e.g., non-governmental organizations (NGOs) and industry), and was approved by the OECD Working Group of the National Coordinators for the Test Guidelines Programme (WNT) at its meeting in April 2023. This guidance includes several case-studies for application of the battery to DNT decision-making. In addition, the guidance includes additional technical characterization of the assays, as it contains appendices that contain a "ToxTemp" form for each assay (Krebs *et al.*, 2019). These "ToxTemp" forms contain information regarding the biological/human relevance, technical performance, appropriate assay positive controls, and domains of applicability for each assay.

DNT NAM Battery Analysis Methods

Assay screening data from ORD and international collaborators were evaluated for activity changes using the publicly available concentration response modeling software ToxCast Analysis Pipeline (tcpl) R package (version 2.1.0). The ToxCast database contains high throughput and medium throughput data for chemicals of interest to US EPA. Concentration response data are normalized, curve-fit, and visualized using tcpl and potency values of activity changes are estimated. In brief, the chemical concentration-response data were normalized on a plate-by-plate basis to the median of the control wells and curve-fit using tcpl. Curve-fitting was performed in the increased bioactivity (up) or decreased bioactivity (down) direction for each assay endpoint, with a few exceptions where the biology precluded a bi-directional response. The 2020 Agency Issue Paper included 34 MEA NFA endpoints (17 endpoints fit in the up and down direction), while the present analysis excluded the 17 'up' endpoints based on recent findings that these endpoints are predominantly inactive and have yet to be validated with positive controls (Carstens *et al*., 2022). The efficacy cutoff was defined as three times the baseline median absolute deviation (BMAD), where BMAD approximates baseline noise using the vehicle control wells and the two lowest concentrations of test chemical wells on each plate, with a few exceptions (please see Table A.1 for additional details). The potency value was indicated by the concentration at 50% maximal activity values (AC_{50}) for any positive assay endpoints in the suite of assays.

In some cases, more than one sample (with three technical replicates) of a compound may have been tested in an assay. While only one sample was tested for malaoxon, two samples were tested in most of the ORD assays for malathion. When positive results were obtained from both samples for the same

[7] https://www.oecd.org/env/ehs/testing/guidance-evaluation-of-data-developmental-neurotoxicity-in-vitro-testing.pdf

endpoint, the mean of the individual sample values was used as the AC_{50} for that endpoint. In cases where the hit call results from two samples of the same compound were not concordant, a number of factors were evaluated to decide how to treat the discordant data. This included consideration of any information regarding sample stability and robustness of positive responses across all endpoints and assays screening with the sample.

True positive responses were determined by several criteria: 1) For all concentration response curves, the goodness of model curve fitting was evaluated using 9 cautionary flags generated in tcpl (Table A.2). Curves with 3 or more flags were considered not reliable (and hence not considered true positive results). The tcpl flags were designed to capture general indicators of excessive noise, borderline activity, or overfitting and may not fully capture the goodness of fit; therefore, expert review was used when necessary. 2) A true positive was also defined by 'selective' activity, or bioactivity occurring below cytotoxicity. Selective activity was computationally defined as a response with a selectivity score of > 0.3 (Stiegler *et al*., 2011; Krug *et al*., 2013; Frank *et al*., 2018). The selectivity score was calculated as the cytotoxicity potency (log10-AC_{50} of the cytotoxicity endpoint for each assay) minus the potency of a positive response (log10-AC_{50}). 3) When a chemical did not demonstrate selective bioactivity, but demonstrated cytotoxicity in the DNT NAMs, a generalized value of cell stress and cytotoxicity-mediated effects of each chemical was considered (i.e., the ToxCast cytotoxicity-associated "burst" value). The "burst" is defined as a lower bound estimate for a concentration that might cause cell stress or cytotoxicity based on a battery of more than 800 *in vitro* assay endpoints (predominantly non-neuronal from high-throughput assays) (Judson *et al.,* 2016). Previous work indicated that chemicals can be cytotoxic at concentrations occurring below the "burst" value (Carstens *et al*., 2022), suggesting that neuronal cell types and/or neurodevelopmental processes can be more sensitive to cytotoxicity compared to generalized *in vitro* cell stress models and should be considered in the interpretation of DNT potential. Therefore, cytotoxic activity in any DNT NAMs occurring below the "burst" was considered when determining a true positive. Additional information on ToxCast data generation and different levels of data analysis can be found in Table A.2 and the Agency website [8]. 4) Lastly, in order to determine true positive activity across different types of assay endpoints, the data were examined holistically, and an expert review was conducted to assess the robustness of the positive endpoints and determine if they represented genuine bioactivity or spurious responses. For example, Carstens *et al*. (2022) reported that MEA-NFA endpoints are highly correlated and sensitive to chemicals that exhibit *in vivo* DNT. Therefore, a low hit rate in the MEA-NFA assay may be indicative of spurious activity. For more information on DNT NAM technologies and data analysis methods, refer to the 2020 SAP Agency Issue Paper and Carstens *et al*., 2022.

Estimation of average blood concentrations using kinetic modeling

The DNT NAM battery data provides an opportunity to examine the relative sensitivity of potential DNT activity to doses that inhibit AChE. Specifically, average blood concentrations estimated at PODs based on 10% AChE inhibition can be compared to AC_{50} values from the DNT NAM battery to examine whether and to what degree PODs based on AChE inhibition are protective of the NAM-based concentrations. This comparison was possible because both the average blood concentration and DNT NAM battery-based AC_{50} are internal concentrations, and each reflect a different biological endpoint. A kinetic model is required to estimate the appropriate internal concentrations at the AChE-based POD.

[8] https://www.epa.gov/chemical-research/toxcast-data-generation-toxcast-pipeline-tcpl

Similar to the other aspects of risk assessment, a tiered approach should be utilized when selecting an appropriate kinetic model to maximize efficiency, minimize the use of animals, and fit the specific purpose. On the continuum of kinetic models that may be applied for estimating the internal concentration, at one extreme are models that require minimum amount of chemical-specific data (such as the HTTK model). At the other extreme are highly refined PBPK models that require a significant amount of time and resources for development and evaluation (such as the PBPK model used in the revise human health risk assessment for malathion and malaoxon). The amount of data and the type of model necessary for estimating the internal concentrations should depend on the purpose of the application and the degree of uncertainty permissible given that purpose.

For most chemicals, PBPK models are not available; however, for malathion/malaoxon, rat and human specific PBPK-PD models were developed to estimate species-specific PODs based on a maximum red blood cell (RBC) AChE inhibition of 10% for the human health risk assessment. Details of this model and its application to derive PODs are described in the revised draft risk assessment to support Registration Review for malathion (W. Britton, Task Group No: 00491975, 01/22/2024). Given the availability, the same model was also used in the current evaluation to obtain the average blood concentrations for comparison with DNT NAM battery-based AC_{50} values. This refined model provides an opportunity to simulate oral (single dose and steady state[9]), dermal, and inhalation exposures. Since refined PBPK models are not available for most chemicals, the HTTK model was also used to predict the average blood concentrations as a proof-of-concept exercise to demonstrate the use of the two modeling approaches, but the comparison of the results may not be generalizable to other chemicals. Details of the HTTK model can be found in the 2020 SAP Agency Issue Paper and peer reviewed literature (Pearce *et al*., 2017; Linakis *et al*., 2020). Currently, the HTTK model can be used to simulate oral and inhalation routes. It is not possible to simulate dermal exposure with HTTK because this exposure route is not currently included in the models.

The average blood concentrations were predicted using the refined PBPK or the HTTK models given AChE-based PODs. PODs were either predicted using the PBPK-PD model based on 10% RBC AChE inhibition or obtained from the previous malathion risk assessment (S. Shalu; 09 SEP-2016; D414107), where benchmark dose (BMD) modeling of AChE inhibition data from animal studies was used to obtain BMD_{10} and $BMDL_{10}$ values[10].

Results

DNT NAM In Vitro Battery

A summary of the DNT NAM battery results is presented in Table 1. The results for each OP compound tested are discussed in more detail below. Additional details and individual plots are also provided in Appendix A to C.

[9] OPs exhibit a phenomenon known as steady state AChE inhibition where the degree of inhibition comes into equilibrium with the production of new, uninhibited enzyme after repeated dosing at the same dose level.

[10] BMD_{10} = estimated dose where AChE is inhibited by 10% compared to background. $BMDL_{10}$ = lower confidence bound of BMD_{10}.

Table 1. Summary of assay endpoints measured and true positive activity for malathion and malaoxon in human and rat neuronal cell lines.

Chemical	Activity type	Assay	Species/Cell line name	# True Positive/ # Total Endpoints Measured	Range of AC_{50} (µM)	Cytotoxicity AC_{50} (µM)	ToxCast "burst" (µM)
Malathion	Proliferation	NPC1	Human (hNPC)	0/6	-	-	9.7
		HCI	Human (hNP1)	0/2	-	-	9.7
	Apoptosis	HCI	Human (hNP1)	0/1	-	41.1	9.7
	Migration	NPC2	Human (hNPC)	0/8	-	-	9.7
	Neuronal Differentiation	NPC3	Human (hNPC)	0/2	-	-	9.7
	NOG	NPC4	Human (differentiated from hNPC)	0/4	-	-	9.7
		HCI	Human (hN2)	0/3	-	-	9.7
		HCI	Human (CDI)*	1/3	16.1[a]	-	9.7
			Human iCell GABA**	2/3	38.3 - 42.0	81.8	9.7
	Oligodendrocyte differentiation	NPC5	Human (oligodendrocyte progenitor)	0/2[b]	-	-	9.7
	Network formation and function	MEA	Rat (primary rat cortical)	14/17	0.80 – 11.4	20.0	9.7
	NOG	HCI	Rat (primary rat cortical)	3/3	12.37 - 85.4	41.2	9.7
	Synaptogenesis	HCI	Rat (primary rat cortical)	3/7	32.56 – 35.15	68.0	9.7
Malaoxon	Proliferation	NPC1	Human (hNPC)	0/6[c]	-	-	-
		HCI	Human (hNP1)	0/2	-	-	-
	Apoptosis	HCI	Human (hNP1)	0/1	-	-	-
	Migration	NPC2	Human (hNPC)	0/8	-	-	-
	Neuronal Differentiation	UKN2	Human (HCS)	0/2	-	-	-
		NPC3	Human (hNPC)	0/2	-	-	-
	NOG	UKN5	Human (SBAD2: primary sensory neurons)	0/2	-	-	-
		UKN4	Human (LUHMES: differentiating dopaminergic neurons)	0/2	-	-	-
		HCI	Human (hN2)	0/3	-	-	-
		NPC4	Human (differentiated from hNPC)	0/4[d]			
		HCI	Human (CDI)*	0/3	-	-	-
			Human iCell GABA**	0/3			
	Oligodendrocyte differentiation	NPC5	Human (oligodendrocyte progenitor)	0/1	-	-	-
	Network formation and function	MEA	Rat (primary rat cortical)	0/17	-	-	-
	NOG	HCI	Rat (primary rat cortical)	0/3	-	-	-
	Synaptogenesis	HCI	Rat (primary rat cortical)	0/7	-	-	-

MEA = microelectrode array network formation assay; HCI = high-content imaging; hNP1= human neural progenitor cell line; NPC1-4 = human primary neuroprogenitor cells (Lonza) in neurosphere cultures & NPC5 = human glial cells in neurosphere cultures developed at Leibniz Institute for Environmental Medicine (IUF). UKN2 = human neural crest from h9 embryonic stem cells; UKN4 = Lund human mesencephalic human embryonic neuronal precursor (LUHMES) cells; UKN5 = human peripheral nervous system cells (immature dorsal root ganglion) cells from h9 embryonic cells, developed at University of Konstanz (UKN).

* At the time of this memo, the NOG (CDI) assay endpoints were labeled 'CCTE_Mundy_HCI_CDI_NOG' in the CompTox dashboard (invitrodb v3.5). In the next release of the dashboard (invitrodb v4.0+), these assay endpoints will have the label 'CCTE_Mundy_HCI_iCellGluta_NOG'.

** At the time of this memo, data from the NOG (iCell GABA) were not reported in the CompTox dashboard. The data are available at doi: 10.23645/epacomptox.22149200 and will be included in the next CompTox dashboard data update with the endpoint labels 'CCTE_Mundy_HCI_iCellGABA_NOG' (estimated release in summer 2023).

[a] Although the CCTE_Mundy_HCI_CDI_NOG_NeuriteLength_loss endpoint demonstrated true positive activity, expert review determined that the activity was a result of poor model fitting. The AC_{50} value for the endpoint CCTE_Mundy_HCI_CDI_NOG_NeuriteCount_loss was determined to be a more accurate estimate of activity changes occurring in the NOG (CDI) assay and was flagged for demonstrated activity only at the highest concentration.

[b] IUF_NPC5_oligodendrocyte_differentiation_120hr_up activity was active however expert review determined the replicate datato be noisy and was therefore not considered a true positive.

[c] IUF_NPC1a_proliferation_BrdU_72hr_dn endpoint was active however expert review determined the activity to be borderline and the replicate data to be noisy and therefore is not a true positive.

[d] IUF_NPC4_neurite_length_120hr_dn endpoint was active however expert review determined the replicate data to be noisy and the result of overfitting of the gain-loss model and was therefore not considered a true positive. IUF_NPC1_viability_72hr_up endpoint was active however this activity was considered noisy and borderline and therefore was not considered active.

Malathion: Using human cells in multi-concentration screening, malathion did not show any true positive responses in assays measuring proliferation, neuronal migration, oligodendrocyte differentiation, apoptosis, neuronal differentiation, and NOG in NPC4 or hN2 cell models. Activity below cytotoxicity was observed in the NOG assays in the human CDI cells; however, this activity was borderline at the highest concentration tested (100 µM) leading to low confidence in the obtained AC_{50} values. Activity below cytotoxicity was also observed in the iCell GABA cell model and were flagged for activity only occurring at the highest concentration tested (100 µM). Using rat cortical cells, malathion showed true positive responses in 14 out of 17 endpoints measuring network formation and function activity, 3 out of 3 endpoints measuring NOG, and 3 out of 7 endpoints measuring synaptogenesis. When positive results were obtained from both samples for a single DNT endpoint, the mean of the individual sample values was used as the AC_{50} for that endpoint. Please refer to Appendix A.2 for additional details.

Malaoxon: There were no true positive responses for malaoxon in rat or human cells. Although there was activity observed in the NOG (NPC4 neurite length) and proliferation (NPC1 viability and BrdU) assays using human cell lines, this activity should be interpreted with caution. The concentration-response curves for these assays were either equivocal or demonstrated inconsistencies between the test replicates. Given this and the low activity of malaoxon in the DNT battery overall, there is low confidence that the activity in these two assays is indicative of true positive results. Please refer to Appendix A.3 for additional details.

Comparing NAM and AChE-based internal concentrations

Since there were no true positive responses for malaoxon in the DNT battery, using either human or rat cells, no average blood concentrations were estimated for malaoxon. No human average blood concentrations were estimated for malathion because malathion activity in the human cell lines occurred at concentrations above the ToxCast "burst". For rat assays with malathion, the lowest AC_{50} value in this set of positive results (minimum AC_{50}: 0.802 µM) was observed in the MEA-NFA and was

substantially lower than the other observed AC_{50} values. Despite its inconsistency with the rest of the battery, including the other assay results in the MEA-NFA, this low value was still conservatively included in the calculation of the average AC_{50} values at this time. When an endpoint exhibited positive hits for both samples, the two AC_{50} values were averaged (see Table A.3 in Appendix). For endpoints with only a positive hit for one sample, no averaging was performed and the value from the positive hit was used as is. The endpoints were then grouped into assay categories by activity type (i.e., network formation & function, NOG, and synaptogenesis) and a median was calculated for each category. The lowest of these median values (11.49 µM) was selected as the point of comparison with the average malathion concentration in rat blood (µM) estimated by the kinetic models that would be associated with 10% AChE inhibition (Table 2).

Table 2. Median AC_{50} Values from DNT NAM Battery for Malathion by Activity Type

Activity Type	Assay Endpoint Name	Assay Endpoint AC_{50} or Mean AC_{50} (µM)[a]	Activity Median (µM)
NOG	HCI_Cortical_NOG_BPCount_loss	48.89	49.50
	HCI_Cortical_NOG_NeuriteCount_loss	52.82	
	HCI_Cortical_NOG_NeuriteLength_loss	50.11	
	HCI_Cortical_NOG_NeuronCount_loss	41.22	
Synaptogenesis	HCI_Cortical_Synap&Neur_Matur_BPCount_loss	33.20	33.20
	HCI_Cortical_Synap&Neur_Matur_NeuriteLength_loss	35.16	
	HCI_Cortical_Synap&Neur_Matur_NeuriteSpotCountPerNeuron_loss	32.82	
	HCI_Cortical_Synap&Neur_Matur_NeuronCount_loss	67.99	
	HCI_Cortical_Synap&Neur_Matur_SynapseCount_loss	32.57	
Network formation & function	MEA_dev_ Alamar blue _dn	24.4	**11.49**[b]
	MEA_dev_ LDH _dn	22.51	
	MEA_dev_active_electrodes_number_dn	11.70	
	MEA_dev_burst_duration_mean_dn	13.17	
	MEA_dev_burst_rate_dn	11.61	
	MEA_dev_bursting_electrodes_number_dn	8.45	
	MEA_dev_correlation_coefficient_mean_dn	11.49	
	MEA_dev_firing_rate_mean_dn	9.55	
	MEA_dev_inter_network_spike_interval_mean_dn	10.33	
	MEA_dev_interburst_interval_mean_dn	12.56	
	MEA_dev_mutual_information_norm_dn	5.63	
	MEA_dev_network_spike_duration_std_dn	10.09	
	MEA_dev_network_spike_number_dn	11.67	
	MEA_dev_network_spike_peak_dn	10.69	
	MEA_dev_per_burst_interspike_interval_dn	15.25	
	MEA_dev_per_burst_spike_percent_dn	13.71	
	MEA_dev_per_network_spike_spike_number_mean_dn	10.70	
	MEA_dev_per_network_spike_spike_percent_dn	11.31	
	MEA_dev_spike_duration_mean_dn	9.35	

[a] Average AC_{50} calculated when positive results were obtained from both samples for a single DNT endpoint.

[b] The bolded value represents the value used as the point of comparison with the average malathion concentration in rat blood (µM) estimated by the kinetic models.

Four exposure scenarios were simulated to estimate average blood concentration using the refined rat PBPK model, including two oral scenarios, one dermal scenario, and one inhalation scenario. Simulated oral scenarios included a single oral dose administered daily over 32 days to reflect steady-state and an

'acute' oral scenario reflecting a single bolus dose at time zero. The oral dosing scenarios were constructed to reflect the acute and steady state *in vivo* studies, which were performed with single or repeated gavage doses of malathion (MRID 45566201, 46822201, 47373704). Additionally, inhalation (6 hours day^{-1}, 5 days $week^{-1}$, modeled for 32 days) and dermal (6 hours day^{-1}, 7 days $week^{-1}$, modeled for 32 days) scenarios were conducted (Table 3). All steady state scenarios were simulated for 32 days to ensure that exposure occurred on the final day (inhalation exposure occurred on 5 out of 7 days of the week). For the dermal scenario, a dermal absorption factor (DAF) of 10.7% over 24 hours was used based on the *in vivo* dermal penetration study in rats (MRID 50974501).

The *in vivo* BMD values for adult animals were used to run steady state oral and inhalation scenarios as outlined above (Table 3). For acute oral exposure, the lowest observed adverse effect level (LOAEL) of 450 mg/kg based on 17% AChE inhibition in adult female rats was used because a BMD_{10} could not be obtained from the available data. The dermal scenario was run using the AChE-based POD predicted for rats by the PBPK-PD model because there was no *in vivo* data available in rats (Table 3). For all modeled scenarios, the average malathion concentration in blood during the final 24 hours that exposure occurred (i.e., day 32 for the inhalation, dermal, and steady state oral scenarios, day 1 of the acute oral scenario) were simulated for comparison with the *in vitro* AC_{50} value.

The refined PBPK predicted average blood concentration for the steady state oral exposure scenario in rats at the *in vivo* BMD_{10} value was ~4800 times lower than the DNT NAM-based AC_{50} value, whereas the refined PBPK predicted average blood concentration for the acute oral scenario at the *in vivo* LOAEL was ~300 times lower than the DNT-based AC_{50} value (Table 3). The average blood concentration for the dermal exposure scenario in rats at the PBPK-PD predicted POD was ~6 times lower than the DNT-based AC_{50} value. The average blood concentration for the inhalation exposure scenario at the *in vivo* BMD_{10} value was also ~6 times lower than the DNT-based AC_{50} value (Table 3). The predicted average blood concentrations were substantially lower than the median values for all rat assay categories (i.e., network formation & function, NOG, and synaptogenesis, Figure 2).

In addition to the refined PBPK-PD model, the HTTK model was also used to estimate average blood concentrations for two oral scenarios (single dose for acute and a daily dose over 32 days for steady state) and a single inhalation scenario (6 hours/day, 5 days/week, 32 days). As with the refined PBPK model estimates, the scenarios in HTTK estimated the average malathion concentration in blood during the final 24 hours that exposure occurred (i.e., day 32 for the inhalation and steady state oral scenarios, day 1 of the acute oral scenario). As above, the average blood concentrations were estimated by simulating exposure at the AChE-based POD (Table 3). The value of key parameters (partition coefficients, Cl_{int}, FUP, and inhalation rate) for the HTTK models were obtained using the same sources as were used for the refined PBPK model.

The average blood concentration estimated using the HTTK model were similar to but lower than those estimated by the refined PBPK model for steady state oral and inhalation scenarios (Table 3) with the greatest difference observed in the acute oral exposure predictions. The difference between the estimates obtained with HTTK and those from the refined PBPK model are attributed to non-linear metabolism and competitive inhibition of malathion metabolism by malaoxon that were only described in the refined PBPK model. The magnitude of the difference driven by these two characteristics of the model would be expected to increase with increasing external dose, which supports that the acute scenario exhibits the greatest difference between the two models. Importantly, the refined PBPK

model was extensively fit to *in vivo* data, which likely confers a greater degree of fidelity to biological phenomena than the predictive models in HTTK.

Table 3. Predicted internal dose metrics for rat exposure to malathion at the AChE-based POD using HTTK and the PBPK-PD model.

Exposure Route	Exposure Scenario	BMD_{10} (mg/kg/day) for AChE from last DRA	Refined PBPK Predicted Average Blood Concentration (µM)[a]	HTTK Predicted Average Blood Concentration (µM)[a]	Fold Difference Between Median AC_{50} and Refined PBPK Blood Concentration[b]
Oral	Acute	450[c]	0.039	0.00068	294
Oral	Steady State 32 days	25[d]	0.0024	0.00038	4787
Inhalation	6 hours day^{-1} 5 days $week^{-1}$ 32 days	53[e]	2.1	0.91	5.5
Dermal	6 hours day^{-1} 7 days $week^{-1}$ 32 days	2200[f]	1.8	NA[g]	6.4

[a] The "predicted average blood concentration" is the average concentration of malathion in the blood during the final 24 hours of exposure (i.e., day 32 for steady state exposures or day 1 for acute exposures).

[b] The fold difference was calculated as the AC_{50} from the DNT NAM battery (11.49; Table 2) divided by the average blood concentration determined using the refined PBPK model.

[c] The AChE-based POD used for simulations for acute oral exposure was the lowest observed adverse effect level (LOAEL) based on 17% AChE inhibition in adult female rats (MRID 45566201). This value was selected because the refined PBPK rat model simulates adult individuals and available BMD_{10} value from the 2016 DRA was from studies using PND11 rat pups (S. Shelat, 06/09/2016, D414107).

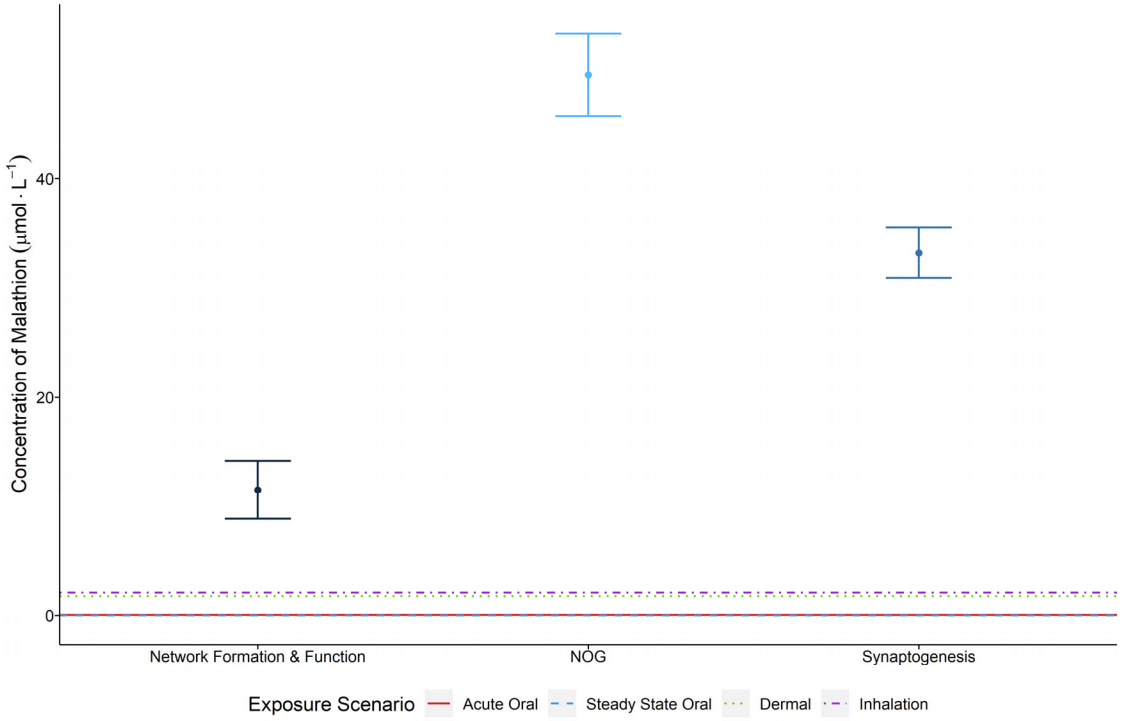
[d] The value used for simulations of steady state oral exposure was the mean BMD_{10} from *in vivo* steady-state oral exposure (S. Shelat, D414107, 06/09/2016).

[e] The BMD_{10} from the 90-day inhalation study in male rats (0.17 mg/L/day) was converted from a concentration in inhaled air (0.17 mg/L) to a dose to match the units in dermal and oral exposures (0.17 mg/L × 12.9 L/hr × 6 hrs/day ÷ 0.25 kg, where 12.9 L/hr was the modeled rat respiration rate, 6 hrs/day was the exposure duration in the study, and 0.25 kg was the modeled rat bodyweight).

[f] Since the available BMD_{10} from the 2016 DRA was determined from a rabbit *in vivo* study, the AChE-based POD used for simulations was predicted using the rat PBPK-PD model.

[g] HTTK does not include a dermal exposure pathway, thus an internal dose metric for dermal exposure could not be determined using this method.

Figure 2: **Predicted average blood concentrations at AChE-based PODs for each exposure scenario compared with the median AC_{50} values for the rat DNT assay categories (i.e., network formation & function, NOG, and synaptogenesis).** Simulations were conducted with the refined rat PBPK-PD model. Error bars represent the interquartile range of the endpoints in each category.



In vivo Studies

In the guideline DNT study in CD rats for malathion (MRID 45646401: 0, 5, 50 and 150 mg/kg/day), no treatment-related functional observations were noted in maternal animals evaluated on gestation days 12 and 18 and lactation days 4 and 10. Clinical signs were limited to transient post-dosing salivation (5/24 in control animals, 4/24 at 5 mg/kg/day, 3/24 at 50 mg/kg/day, and 20/24 at 150 mg/kg/day). Although the study authors attributed the increase in post-dosing salivation to distaste of the formulation, this effect was conservatively considered treatment related by the Agency. As a result, the maternal no observed adverse effect level (NOAEL) was established at 50 mg/kg/day and the LOAEL at 150 mg/kg/day based on an increased incidence of post-dosing salivation.

In the offspring, there were no differences among treatment groups with respect to pup survival, body weight or food consumption, day of sexual maturation, learning and memory evaluations, or brain weights. Four offspring in the 150 mg/kg/day group exhibited whole body tremors and hypoactivity after dosing on PND 17 and 18. Two of these pups also exhibited prostrate posture and partially closed eyelids on PND 17 and another of these pups showed abnormal gait on PND 19. Additionally, in the functional observational assessment, the mean surface righting score for PND 11 female pups at 150 mg/kg/day was increased (1.6) as compared to control females (1.0). At lower doses, observations were limited to transient post-dosing salivation in one pup at each dose. Therefore, the offspring NOAEL was established at 50 mg/kg/day with a LOAEL of 150 mg/kg/day, based on clinical signs (whole

body tremors, hypoactivity, prostrate posture, partially closed eyelids) in males and females, and delayed surface righting reflex in PND 11 female pups.

Cholinesterase measurements were not performed in the DNT study; however, there are numerous studies available in the malathion database that demonstrate considerable AChE inhibition would have been observed if AChE measurements had been included in the DNT study. For example, in a companion comparative cholinesterase study with malathion (MRID 45566201), approximately 20% RBC cholinesterase inhibition was demonstrated at 50 mg/kg/day in maternal animals dosed by gavage from GD 6-20, and in young adult rats that were dosed by gavage for 11 consecutive days. At 150 mg/kg/day, 51% RBC cholinesterase inhibition was observed in maternal animals, and 43-48% RBC cholinesterase inhibition was observed in young adults. In pups, following a single dose on PND11, effects on RBC cholinesterase were observed at the lowest dose tested of 5 mg/kg, with greater inhibition observed in males (16% in males vs. 7% in females). Inhibition of RBC cholinesterase increased with increasing dose and male pups demonstrated similar or slightly increased inhibition as compared to female pups. RBC inhibition was observed in PND21 pups at ≥5 mg/kg/day following repeated exposures with no apparent sex differences (15% in males vs. 17% in females at 5 mg/kg/day). Therefore, significant AChE inhibition was occurring at and below doses that elicited either maternal or offspring toxicity in the DNT study.

Additionally, as part of the literature search for malathion, another study was identified that examined the neurobehavioral effects of malathion exposure at doses of 5 or 15 mg/kg (Ouardi et al., 2019). Although this study was not evaluated in detail (W. Britton, Task Group No: 00491975, 01/22/2024), the observations by the study authors occurred in the presence of substantial AChE inhibition (12-24% in pups of 5 mg/kg malathion-treated dams; greater inhibition at 15 mg/kg).

Epidemiology Studies

HED conducted an extensive search of the literature in 2015 to identify epidemiological investigations of the association between OP exposures and potential DNT outcomes (A. Lowit, D331251, 15-SEP-2015). The review was then updated in 2016 to incorporate additional studies and address public comments (A. Aldridge et al., D437043, 29-DEC-2016). Exposure in most of these studies was assessed using biomarkers. In limited instances, exposure was assessed using a direct measure of an OP pesticide (e.g., chlorpyrifos measures in blood) or a metabolite specific to a particular OP. In the case of malathion, there was one study identified (Wolff et al., 2007) that evaluated malathion exposures using a metabolite specific to malathion malondialdehyde (MDA), which focused solely on birth outcomes (birth weight, length, ponderal index, head circumference, and gestational age) and found no associations.

The majority of the epidemiology studies in the 2015/2016 review used non-specific biomarkers of OP exposure, with urinary dialkyl phosphates (DAPs) being the most commonly measured biomarkers. DAPs are considered non-toxic metabolites[11], and each DAP is a breakdown product from multiple OPs, making it impossible to separate exposure and associated effects for individual, specific OPs. Another limitation associated with using urinary DAPs as biomarkers as an exposure measure includes temporal

[11] https://www.cdc.gov/biomonitoring/OP-DPM_FactSheet.html#:~:text=Once%20they%20enter%20the%20body,an%20exposure%20to%20organophosphate%20insecticides

variability, with levels often varying substantially over short time scales (i.e., day-to-day). As such, quantification of DAPs in a single urine sample may not represent an individual's typical exposure to OP pesticides since it only represents a "snapshot" in time that can underestimate or overestimate typical exposures. Additionally, urinary DAP levels may also reflect direct exposure to DAPs, rather than exposure to an OP, because DAPs can form through degradation processes in/on food and in the environment. For example, DAPs can be present in commodities prior to consumption (e.g., Chen et al., 2012; Zhang et al., 2008). Therefore, measured DAP levels can be a reflection of OP exposure, direct DAP exposure (e.g., through consumption of commodities containing DAPs), or a combination thereof. Furthermore, multiple FIFRA SAPs have identified uncertainties in the epidemiological data[12], including but not limited to relatively modest sample sizes, concerns with the representativeness of a single point exposure, potential for exposure misclassification, and questions about "biologic plausibility due to lack of clarity on mechanism of action". As a result, although the measures in the epidemiological studies involving DAPs may provide qualitative evidence that exposure to one or more OPs occurred, the actual level of such exposure during critical window(s) of susceptibility is not known. Consequently, these studies are unable to provide a robust quantitative evaluation of the sensitivity of potential DNT effects relative to AChE inhibition for any specific OP.

This WOE evaluation focused on those epidemiology studies that report chemical-specific effect estimates for the association between malathion exposure and health outcomes that are potentially DNT related. In 2021, in support of the Registration Review risk assessment for malathion, HED conducted a review of the peer-reviewed epidemiology literature with the aim of identifying epidemiological studies that reported effect measures *specific* to associations between malathion exposure and health outcomes, including potential DNT outcomes (A. Aldridge et al., D462819, 30-MAR-2021). The epidemiology studies assessed within this memo included direct exposure of malathion including some studies that were able to measure the malathion specific metabolite, malondialdehyde (MDA), using urinary and/or blood concentrations. The 2021 review used the methods described in OPP's "Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides[13]", the methods described in Sections 3.0-3.5 of the *Malathion: Tier II Review of Human Incidents and Epidemiology Review*, and generally followed the guidance provided by the National Toxicology Program/Office of Health Assessment and Translation (NTP/OHAT)[14]. The 2021 literature review considered publications available in peer-reviewed literature databases (PubMed, PubMed Central, Scopus, and Science Direct) and a HED-maintained electronic library of published articles from the Agricultural Health Study (AHS)[15], a prospective cohort study of farmer pesticide applicators and their families in Iowa and North Carolina of the United States. Five publications from the 2021 review investigated a potential DNT outcome and are considered part of the epidemiology evidence in this WOE evaluation.

[12] Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting held April 10-12, 2012 on "Chlorpyrifos Health Effects" - https://www.regulations.gov/document/EPA-HQ-OPP-2012-0040-0029
Transmittal of Meeting Minutes of the April 19-21, 2016 FIFRA SAP Meeting Held to Consider and Review Scientific Issues Associated with "Chlorpyrifos: Analysis of Biomonitoring Data" - https://www.regulations.gov/document/EPA-HQ-OPP-2016-0062-0140

[13] US EPA. December 28, 2016. Office of Pesticide Programs' Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides. https://www3.epa.gov/pesticides/EPA-HQ-OPP-2008-0316-DRAFT-0075.pdf

[14] Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration, January 9, 2015.

[15] https://aghealth.nih.gov/

Since the 2021 epidemiology review for malathion, HED identified six additional publications that evaluated malathion exposure and potential DNT outcomes. A detailed summary and review of the strengths and limitations for each of the six additional publications is provided in the *Malathion: Review of Epidemiology Studies on the Association between Malathion Exposure and Neurodevelopmental/Neurobehavorial Outcomes* memorandum (A. Aldridge et al., Task Group No: 00491986, 22-JAN-2024).

In total, HED has identified eleven publications that reported on the potential association between malathion exposure and potential DNT outcomes in six study populations including 1) the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) longitudinal birth cohort of pregnant women and their children in a farmworker community in the Salinas Valley of California (Eskenazi et al., 2007; Gunier et al., 2017; Sagiv et al., 2018; Hyland et al., 2021; Hyland et al., 2022; Gunier et al., 2022); 2) a population-based study of children living in an agricultural area in San Joaquin Valley of California (von Ehrenstein et al., 2019); 3) a registry-based case-control study in California (Rull et al., 2006); 4) a cohort of pregnant women and their children in the Children's Environmental Health longitudinal study, part of Mount Sinai hospital in New York City, New York (Engel et al., 2007); 5) a population based case-control study of mothers and their children living in the state of California (Liew et al., 2020), and 6) a cross-sectional study of adolescent boys, part of the Environment and Childhood (INMA) cohort, in Granada, Spain (Rodriguez-Carrillo et al., 2022).

The epidemiology evidence primarily included studies that tested for neurodevelopmental effects in children using psychometric tests and neurodevelopmental and behavioral outcomes. Neurodevelopmental behaviors or traits are typically grouped into meaningful categories called domains that focus on a particular kind of activity and roughly map onto functioning of specific brain regions. Several brain regions may be involved in the behavior. Additionally, neurodevelopmental traits such as attention, are not isolated attributes, they interact with other domains in determining behavior. General domains include attention, executive function, motor function, learning and memory, social-emotional, verbal/language, visuospatial function, and processing speed; and each domain may have several subdomains of neurobehavioral function (White *et al*., 2022).

Psychometric tests are typically used to estimate potential effects of toxicants on the nervous system and are used in epidemiology to assess facets of neurodevelopment. Importantly, a null result on a psychometric test may not indicate lack of neurotoxicity of the chemical being investigated. The null result could indicate that the toxicant does not affect the biological pathways related to the domain, subdomain, or outcome being measured by that psychometric test chosen for the study (White *et al*., 2022).

For this review, investigated health outcomes of the epidemiology studies were grouped into the following neurodevelopmental domains: learning and memory, general intelligence/IQ, executive functioning, processing speed, attention, clinical conditions (autism spectrum disorder with and without intellectual disability), social-emotional (anxiety, depression, hyperactivity, and internalizing and externalizing behaviors, behavioral function), and developmental (the number of abnormal reflexes). Additional health outcomes in this evaluation that were related to neurodevelopmental effects included neural tube defects, risk taking behaviors in adolescents, and cerebral palsy. See Appendix D for a tabular summary of design elements of each study.

The CHAMACOS cohort used the Behavior Assessment for Children (BASC) psychometric test on 16- and 18-year-old participants to gather information about malathion exposure and possible effects in the social-emotional domain of neurodevelopment (Hyland et al., 2021 and 2022). In a separate study, the CHAMACOS cohort used the Bayley Scales of Infant Development (MDI and PDI Indices) and the Child Behavior Checklist (CBCL) psychometric tests on infant children at 6, 12, and 24-months of age to investigate malathion exposure and effects in the developmental and social-emotional neurodevelopmental domains (Eskenazi et al., 2007). In another study, Gunier et al., 2017, the CHAMACOS cohort used the Wechsler Intelligence Scale for Children (WISC) psychometric test to evaluate malathion exposure and domain-specific intelligence quotient (IQ) (Working Memory, Processing Speed, Perceptual Reasoning, Verbal Comprehension) among 7-year-old children. The investigators from the Environment and Childhood (INMA) Project, a birth cohort study in Granada, Spain (Rodriguez-Carrillo et al., 2022) used the Child Behavior Checklist (CBCL, Spanish version) on male adolescents to evaluate malathion exposure and effects in the social-emotional domain of neurodevelopment. The Mount Sinai Children's Environmental Health cohort study used the Brazelton Neonatal Behavioral Assessment Scale (BNBAS) psychometric test on neonates to investigate malathion exposure and possible effects in the developmental domain of neurodevelopment (Engel et al., 2007). The investigators of the CHAMACOS cohort (Sagiv et al., 2018) used the Social Responsiveness Scale (SRS-2), the Behavior Assessment System for Children (BASC), the Evaluación Neuropsicológica Infantil (ENI) Facial Expression Recognition Test[16], and the NEPSY-II Affect Recognition subtest[17] to investigate the association between malathion exposure and the neurodevelopmental disorder, autism spectrum disorder, associated with behaviors predominantly part of the social-emotional domain. Additionally, a population-based study (von Ehrenstein et al., 2019) used data from California statewide registries to assess the potential association between malathion exposure and autism spectrum disorder with or without intellectual disability of children living in an agricultural area in San Joaquin Valley of California, using the Diagnostic and Statistical Manual of Mental Disorders (DSM).

Additional outcomes that were not assessed using psychometric tests included risk-taking behaviors and delinquent acts, neural tube defects, and cerebral palsy. Gunier et al. (2022) used a questionnaire adapted from the Self-Reported Behavior and Self-Reported Delinquency scales to assess delinquency and risk-taking behaviors in youth in the CHAMACOS cohort. And the two remaining publications used data from California statewide registries on developmental disabilities and birth defects to assess the potential association between malathion exposure and cerebral palsy (Liew et al., 2020) and neural tube defects (Rull et al., 2006) among residents of California.

[16] The TENI (Test de Evaluación Neuropsicológica Infantil) is a tool used to assess neuropsychological abilities in children and has been tested for psychometric-like test properties. Matute, E., Rosselli, M., Ardila, A., & Ostrosky-Solís, F. (2007). Evaluación neuropsicológica infantil. *México: Manual Moderno*. Martins, P. S., Barbosa-Pereira, D., Valgas-Costa, M., & Mansur-Alves, M. (2022). Item analysis of the Child Neuropsychological Assessment Test (TENI): Classical test theory and item response theory. *Applied Neuropsychology: Child*, *11*(3), 339-349.

[17] The NEPSY-II Affect Recognition subtest is an instrument used to determine neuropsychological development in children and has been tested for psychometric-like test properties. Korkman, M., Kirk, U., & Kemp, S. (2007). NEPSY—Second Edition (NEPSY-II); San Antonio, TX. *J. Psychoeduc. Assess*, *28*, 175-182. Yao, S. Y., Bull, R., Khng, K. H., & Rahim, A. (2018). Psychometric properties of the NEPSY-II affect recognition subtest in a preschool sample: a Rasch modeling approach. *The Clinical Neuropsychologist*, *32*(1), 63-80.

Regarding the exposure assessment of the relevant epidemiology studies, authors of the cohort of mother-infant pairs (Engel et al., 2007; Eskenazi et al., 2007) and the cross-sectional study involving male adolescents (Rodriguez-Carrillo et al., 2022) directly measured exposure to malathion through its specific metabolite, MDA, using spot urine samples. However, most studies relied on a GIS-based exposure assessment method that estimated exposure based on geographic proximity of the pregnant mother's residence to treated agricultural fields. Briefly, the GIS-based tool integrates pesticide use reporting data from the California Department of Pesticide Regulation (DPR)[18] and geographic proximity of residences to treated agricultural fields at a distance (usually 1-2 km) that was shown to be most strongly correlated with concentrations of agricultural pesticides in dust samples collected from homes in that region (Harnly et al., 2009; Gunier et al., 2011) to estimate potential pesticide exposure during specific timepoints (i.e., before, during, and after pregnancy).

Certain analyses included the residence reported on the birth certificate and other analyses determined the exact maternal address during prenatal and postnatal periods reducing the uncertainty in the exposure assessment. Using this method and the CA DPR pesticide use reports, the amount of pesticides applied in nearby agricultural fields within a certain radius of the pregnant woman's residence for the duration of the exposure time period (i.e., preconception, pregnancy, first year of life) based on a known address for each trimester or on the address reported on the birth certificate was used to estimate exposure. The address on the birth certificate is less specific than addresses by trimester as it does not account for possible residential mobility during pregnancy[19]. One publication (Gunier et al., 2017) used the GIS-based method to estimate exposure to malathion and other pesticides and then used maternal urinary DAP concentrations as a covariate in the statistical model to adjust for other pesticide exposures. The GIS-based assessments may have limited ability to investigate the relationship between pesticide exposures including malathion and neurodevelopment outcomes if there is a correlation between malathion and other pesticides. As such, these investigations may be unable to distinguish between factors associated with geographic proximity to agricultural land and living, pesticide use in general, and specific pesticides.

Statistically significant associations were reported between malathion exposure and attention problems (Hyland et al., 2021; Hyland et al., 2022), internalizing behaviors and hyperactivity (Hyland et al., 2022), as well as delinquent acts pertaining to risk taking behaviors among adolescents (Gunier et al., 2022) in the CHAMACOS prospective cohort study population, and abnormal reflexes relative to neonatal central nervous system function (Engel et al., 2007) in the Children's Environmental Health prospective cohort study, part of Mount Sinai hospital in New York City, New York. Below are short summaries of the statistically significant associations observed.

- Hyland et al. (2021) reported a borderline statistically significant maternally reported decrease for attention problems among children at 16 and 18 years of age, and a youth-reported decrease for attention problems among girls (not boys) of the same age, following a 2-fold increase in malathion applications within 1 km of the residence during childhood (0-5 yrs). No evidence of a significant

[18] https://www.cdpr.ca.gov/docs/pur/purmain.htm

[19] Past studies have indicated that around 11 – 32 % of pregnant women move their residence at least one time throughout pregnancy, and the median move distances were between 4.2 – 10 km (Lupo et al., 2010; Strickland et al., 2017; Pereira et al., 2016).

association was reported for any other endpoint (hyperactivity, internalizing problems, depression, anxiety, and externalizing problems).

- Hyland et al. (2022) reported statistically significant associations for attention problems, hyperactivity, and internalizing behaviors among children 16-18 years old within the study. For each neurobehavorial outcome, several analyses were conducted, and the data was further stratified in many ways (e.g., maternal vs. youth-reported outcomes, low vs high adverse childhood experiences (ACEs) prenatal vs. childhood exposure, child's sex, age of children at outcome (data assessed at children 16 and 18 years of age vs data assessed at children only 18 years of age). For internalizing problems, a significant increase was consistently observed across analyses for boys (not girls) with high ACEs in both analysis using data assessed at 16 and 18 years old and analysis using only data assessed at 18 years old, following a 2-fold increase in malathion use within a 1 km radius of residence during pregnancy, based on maternal and youth-reported data. A significant youth-reported increase in attention problems for boys (not girls) was reported among children 16 and 18 years old with high ACEs, as well as a significant increase in youth-reported hyperactivity among children 16 and 18 years old (boys and girls) with high ACEs, following a 2-fold increase in malathion use within a 1 km radius of residence during pregnancy. No evidence of a significant association was observed for any other outcome.

- Gunier et al. (2022) reported a slight positive association for delinquent acts (specifically for number of delinquent acts and frequency of delinquent acts) among children with high ACEs at 18 years of age, following a 2-fold increase in malathion use within 1 km of the residence during pregnancy when adjusting for exposure to other pesticides. No evidence of a significant positive association was observed for children with low ACEs, or among all children combined for number of unique delinquent acts and for frequency of delinquent acts at age 18 years, and for any other outcome (police encounters, any delinquent act, or risk count) among all children combined and among children with high or low ACEs at 18 years of age. Without adjustment for exposure to other pesticides, there were no significant associations reported.

- Engel et al. (2007) reported evidence of a moderately strong to strong association between prenatal exposure (given only 21% detection vs. 79% non-detection) and an increased number of abnormal reflexes, part of the central nervous system function, in neonatal babies using the Poisson regression (counts of abnormal reflexes) and multivariable logistic regression model (dichotomized the counts of abnormal reflexes). No evidence of a statistically significant change was observed for any of the other six domains in the multivariable linear model: habituation, orientation, motor performance; regulation of state, range of state, and autonomic stability in newborns following prenatal exposure to malathion.

With the exception of Hyland et al. (2022), the reported positive associations in the three mentioned studies above were not consistently observed across analyses within studies (e.g., different statistical models were used, maternal-reported vs. youth-reported), were only observed following stratified analyses, and/or were not statistically significant or were borderline statistically significant. All of the studies that reported positive associations (except Engel et al., 2007) relied upon a GIS-based assessment approach to estimate pesticide exposure at the maternal residence, instead of directly

measuring exposure. Engel et al. (2007) measured the urinary metabolite of malathion, MDA, using a single urine sample collected once during pregnancy.

As described above, significant increases were reported in Hyland et al. (2022) for internalizing problems among adolescents, particularly for boys, with high ACE with maternal- and youth-reported data and for analyses at 16 and 18 years old and 18 years only. A significant youth-reported increase in attention problems for boys (not girls) was also reported among children 16 and 18 years old with high ACEs following a 2-fold increase in malathion use within a 1 km radius of residence during pregnancy. While Hyland et al. (2021) also reported a borderline statistically significant association for attention problems among children at 16 and 18 years of age following a 2-fold increase in malathion use within a 1 km radius of residence, the exposures were different (pregnant mothers vs children) and the directionality of the reported associations were not the same; Hyland et al. (2022) reported a significant *increase* in attention problems and Hyland et al. (2021) reported a borderline *decrease* in attention problems in children. Although associations were reported in these single studies, Hyland et al. (2021, 2022) studies were part of the same study population, and additional study populations would be needed to evaluate any potential patterns and/or trends in the evidence for internalizing problems, hyperactivity, and attention problems in adolescents.

For neonatal neurodevelopment, a moderately strong to strong association was observed between malathion exposure and the number of abnormal reflexes in one study (Engel et al., 2007). Although an association was observed for the number of abnormal reflexes in neonates, study uncertainties were present that included a single urinary sample taken once during pregnancy to assess malathion exposure, and potential exposure misclassification due the transient and variable nature of exposures to pesticides. Further, the study's statistical methods lacked adjustment for the multiple tests performed, in addition to using an automated backward elimination procedure to determine which confounders/covariates remained. As a result, additional study populations would be needed to evaluate any potential patterns and/or trends in the evidence for the number of abnormal reflexes in newborns.

No evidence of a significant association was reported for six outcomes: neurodevelopmental effects in infants and children (Eskenazi et al., 2007), autism spectrum disorder (with and without intellectual disability) (Sagiv et al., 2018; von Ehrenstein et al., 2019), and learning, memory, intelligence and cognitive development among children (Gunier et al., 2017), all part of the CHAMACOS cohort; neural tube defects in the registry-based case-control study in California (Rull et al., 2006); cerebral palsy in children in a population based case-control study of mothers and their children living in the state of California (Liew et al., 2020); and behavioral function among adolescent boys in a cross-sectional analysis (Rodriguez-Carrillo et al., 2022).

HED evaluated the eleven studies that reported on the association between malathion exposure and health outcomes relevant to the DNT potential WOE evaluation. The eleven studies were either reviewed in the 2021 literature review or in the more recent 2023 memorandum on the additional neurodevelopmental studies. In both reviews, HED concluded that there was *insufficient epidemiological evidence* of a clear associative or causal relationship between malathion exposure and the following DNT outcomes: learning and memory, attention, hyperactivity, and externalizing and internalizing behavior, general intelligence/IQ, social-emotional, and clinical conditions (autism spectrum disorder with and without intellectual disability), the number of abnormal reflexes

(developmental), risk-taking behavior in adolescents, cerebral palsy in children, behavioral function in adolescent boys, and neural tube defects. This conclusion was based on small body of studies (i.e., typically only one or two studies per health outcome) that often had substantive limitations with respect to their exposure assessment approach and/or outcome assessment, a lack of consistent evidence of a positive association, and the potential for bias in the available studies. Additional details including more in-depth discussion of the strengths and limitations of the studies reviewed here can be found in the *Malathion: Tier II Incident and Epidemiology Report* (A. Aldridge et al. D462819, 30-MAR-2021) and in the *Malathion: Review of Epidemiology Studies on the Association between Malathion Exposure and Neurodevelopmental/Neurobehavioral Outcomes* (A. Aldridge et al., Task Group No: 00491986, 22-JAN-2024).

Discussion

As previously mentioned, OPP took a conservative approach by performing the 2015/2016 review for the OPs as a group based on the assumption that, like AChE inhibition and subsequent neurotoxicity, DNT outcomes would share a common MOA/AOP and therefore similar potential DNT concerns would exist across OPs. At that time, the uncertainties in the human dose-response relationship for potential neurodevelopmental outcomes and its quantitative relationship to AChE inhibition prevented reduction of the 10X FQPA SF for the OPs.

Based on the best available science that indicates differences in DNT potential exist across OPs, OPP has determined that DNT potential of OPs should be evaluated on a chemical-by-chemical basis. Therefore, the purpose of this evaluation was to assess the DNT potential of malathion and its metabolite/degradate, malaoxon, using chemical-specific data to evaluate the sensitivity of potential DNT effects relative to AChE inhibition. For this evaluation, the chemical-specific studies available included *in vitro* and *in vivo* assays as well as epidemiology studies. In the case of DNT, exposure to xenobiotics during critical stages of development may result in altered neural development leading to potential lifelong ramifications. Assessing the potential DNT hazard and/or risk from a chemical exposure is a complex process that involves multiple evidence streams that converge in a WOE driven evaluation. Historically, most of the DNT information has been collected in toxicology studies using animal models. However, it is well recognized that any given assay or study, including the *in vivo* DNT guideline study, will not fully evaluate all elements of the nervous system. As such, the strengths and limitations associated with each line of evidence need to be taken into consideration and balanced with those associated with other lines of evidence, including the quality and human relevance of the data obtained from other studies and assays.

The basic purpose of DNT guideline testing in animals is to assess the potential of chemicals to cause adverse neurodevelopmental outcomes. This is achieved through a series of evaluations that measure the functional and/or structural integrity of the developing nervous system. The strengths of the *in vivo* DNT guideline study reside in its ability to evaluate multiple functional domains using a whole organism. Testing with laboratory animals captures intact biological and physiological conditions, including absorption, distribution, metabolism, and interactive biology, in a living system. This includes evaluation of numerous endpoints (functional, behavioral, and anatomical) in the nervous system at multiple time points across different lifestages. However, it does not completely evaluate all aspects of nervous system structure and function/behavior.

In the malathion DNT guideline study, offspring effects (whole body tremors, hypoactivity, prostrate posture, and partially closed eyelids in males and females and delayed surface righting reflex in PND 11 female pups) and maternal effects (increased incidence of post-dosing salivation) were observed at the highest dose tested of 150 mg/kg/day. Because the DNT guideline study infers DNT effects on the basis of apical endpoints, with little or no information on the underlying biological processes responsible for the observed phenotype, it is unknown if the observed effects truly represent developmental toxicity derived from nervous system disruption. Reliable detection, measurement, and interpretation of treatment related DNT effects in the guideline study depends on appropriate study design and conduct that adequately controls for confounding factors such as variability (*e.g.,* due to dosing regimen, age at treatment and assessment, or inherent measure variability), impact of systemic maternal and/or offspring toxicity, experimental procedures, environmental conditions, etc.[20]. Despite these uncertainties, the guideline study, as well as the available literature study that evaluated neurobehavioral effects in laboratory animals, clearly demonstrated that AChE was the most sensitive adverse effect observed.

Epidemiological studies are aimed at investigating associations between a risk factor (e.g., chemical exposure) and particular health outcomes in humans. An obvious strength of an epidemiological study is its evaluation of the relevant species (humans) and groups of interest (e.g., elderly, children, etc.) at relevant exposure levels thereby obviating the need to extrapolate across species or from high exposure levels. Another strength is the ability of epidemiological studies to research a wide range of health outcomes, some of which may be difficult to evaluate in experimental animals or an appropriate animal model may not exist for evaluation. However, major limitations in epidemiological studies are associated with the inability to control the populations and exposures being investigated. Errors in epidemiological studies typically arise from chance, bias, and confounding. Researchers attempt to minimize the impact of these errors through study design and execution (e.g., sufficient sample size, proper selection of study subjects, measurement of potential confounders, statistical analysis methods). Multiple FIFRA SAPs have identified uncertainties in the epidemiological data in the 2015/2016 review[21], including but not limited to relatively modest sample sizes, concerns with the representativeness of a single point exposure, potential for exposure misclassification, and questions about “biologic plausibility due to lack of clarity on mechanism of action”. The limitations of epidemiology studies were considered in the evaluation of the epidemiology evidence on the association between malathion and potential DNT outcomes.

Direct measurement of all exposures of interest, including the chemical under evaluation and potential confounders, can be challenging and resource intensive in environmental epidemiological studies. As a result, information from questionnaires, interviews, or other proxies are frequently used to determine exposure in lieu of direct measurements. Limitations in exposure measurements often make it difficult to utilize the results of epidemiological studies to perform a robust evaluation of dose-response and preclude the use of these data for deriving PODs for risk assessment. As discussed earlier, the

[20] Limitations associated with the DNT guideline have been described in more detail in the 2020 Agency Issue Paper https://www.regulations.gov/document/EPA-HQ-OPP-2020-0263-0006

[21] Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting held April 10-12, 2012 on “Chlorpyrifos Health Effects” - https://www.regulations.gov/document/EPA-HQ-OPP-2012-0040-0029
Transmittal of Meeting Minutes of the April 19-21, 2016 FIFRA SAP Meeting Held to Consider and Review Scientific Issues Associated with "Chlorpyrifos: Analysis of Biomonitoring Data" - https://www.regulations.gov/document/EPA-HQ-OPP-2016-0062-0140

2015/2016 review primarily utilized studies using biomarkers, known as DAPs, that were not specific to any particular OP, including malathion, and may not even reflect exposure to any OPs (e.g., direct consumption of DAPs on commodities). Along with the additional limitations associated with DAPs (e.g., temporal variability, sampling at a single time point), EPA has also noted that studies utilizing DAPs as biomarkers of OPs have not consistently observed associations with potential DNT outcomes. As a result, although the use of DAPs may provide qualitative evidence that exposure to one or more OPs occurred, the actual level of such exposure during critical window(s) of susceptibility is not known and the use of DAPs or other non-specific biomarkers as an exposure measure restricts the ability of the study to inform chemical-specific DNT potential. Similar challenges exist with studies that utilize specific biomarkers of OPs and determining actual levels of exposure during critical window(s) of susceptibility. Furthermore, evaluation of biomarkers requires an understanding of degradation and metabolism of chemicals in both the environment and human body. Differences in metabolism and uncertainty as to whether the biomarker measures exposure to the active ingredient or the environmental degradates may account for apparent differences in biomarkers of exposure among individuals, and possibly between comparison groups.

For this WOE analysis, OPP considered eleven epidemiologic publications that reported on the association between malathion exposure and potential DNT outcomes. The eleven publications reported on several different potential DNT health outcomes, and mixed results were observed across six cohort, case-control, and cross-sectional study populations and eight different neurologic outcomes. Evidence of statistically significant associations were reported between malathion exposure and attention problems (Hyland et al., 2021; Hyland et al., 2022), internalizing behaviors and hyperactivity (Hyland et al., 2022), as well as delinquent acts pertaining to risk taking behaviors (Gunier et al., 2022) among 16-18-year-old children in the CHAMACOS prospective cohort study population, and the number of abnormal reflexes relative to the neonatal central nervous system function (Engel et al., 2007) in the Children's Environmental Health prospective cohort study, part of Mount Sinai hospital in New York City, New York (four of the eleven identified publications). No evidence of a significant association was reported for six outcomes: neurodevelopmental and behavioral effects in infants and children (Eskenazi et al., 2007), autism spectrum disorder (with and without intellectual disability) (Sagiv et al., 2018; von Ehrenstein et al., 2019), and learning, memory, intelligence and cognitive development among children in the CHAMACOS cohort (Gunier et al., 2017); neural tube defects in the registry-based case-control study in California (Rull et al., 2006); cerebral palsy in children in a population based case-control study of mothers and their children living in the state of California (Liew et al., 2020); and behavioral function among adolescent boys in a cross-sectional analysis in the Environment and Childhood (INMA)-Granada cohort in Spain (Rodriguez-Carrillo et al., 2022).

Several challenges were identified that introduced uncertainty in the evaluation of the relationship between malathion exposure and DNT outcomes. Seven of the eleven studies either reported associations that were not significant (e.g., odds ratio (OR) > 1.00, but not statistically significant) or no associations (e.g., for reported beta coefficients (βs), all 95% CIs included the null value of 0) between malathion exposure and DNT health outcomes. Additionally, for three of the four studies that reported a significant association, pesticide exposure was not directly measured, and exposure estimates instead relied on geographic proximity of residence to treated agricultural fields. The GIS-based assessments may have limited ability to investigate the relationship between malathion specifically if there is a moderate correlation across different pesticides applied in that study area. As such, these

investigations may be unable to distinguish between factors associated with geographic proximity to agricultural land and living areas, pesticide use in general, and exposure to specific pesticides. Further, the majority of these health outcomes were investigated in a single study population making it challenging to evaluate patterns or trends in the evidence. As a result, the overall epidemiological evidence is considered insufficient to conclude that there is a clear associative or causal relationship between exposure to malathion and subsequent DNT outcomes investigated in the epidemiology published literature.

In contrast to the guideline and epidemiological studies, the DNT NAM battery is not performed using an intact organism and may lack potentially impactful aspects of a living organism exposure, such as blood-brain and blood-placental barriers, and may have limited metabolic capacity compared to *in vivo* models. The concept of evaluating key neurodevelopmental processes was designed to address the complexity of brain development, given processes must take place across all brain regions and neurotransmitter types for proper nervous system development, and the mechanisms underlying these processes are well conserved. Even though *in vitro* assays do not entirely recapitulate an intact organism, estimates of sensitivity (61-87%) and specificity (71-93%) for different groups of DNT assays indicate that they are capable of detecting effects and discerning between potential type I and type II errors (Harrill et al. 2018; Shafer et al. 2019; Masjosthusmann et al. 2020) and are expected to improve as more chemicals are tested, and the battery continues to evolve. By focusing on these critical biological processes, the DNT NAM battery allows OPP to evaluate potential upstream disruptions that are associated with a spectrum of neurodevelopmental health outcomes. As a result, OPP can protect for a wide range of neurodevelopmental effects by using the DNT battery in a WOE evaluation.

In the 2020 SAP Report[22], the panel agreed that the DNT NAM battery reflects, if not directly models, critical processes of neurodevelopment, but also noted several processes and cell types that were perceived to be missing in the battery. As discussed in the Agency's response to the SAP[23], the current battery is not entirely lacking in these processes and cell types. For instance, although the panel encouraged development and inclusion of glial-based targeted NAMs in the battery, there are several assays in the battery that include glia and allow for potential detection of effects through a glial mediated mechanism despite not specifically measuring glial endpoints. The 2020 SAP also acknowledged that the battery will continue to evolve as the science advances, but this does not preclude the use of data from the battery in a WOE evaluation. Taken together with the recent adoption by the OECD WNT of a guidance document on the use of the battery as part of an IATA for DNT, there is consensus that data from the DNT NAM battery can and should be utilized as part of a WOE evaluation.

It is within the challenges associated with the *in vivo* DNT guideline and epidemiology studies that the value of NAMs become evident. Integrating *in vitro* and computational information with available *in vivo* and epidemiology data as part of an overall WOE evaluation can address some of the limitations encountered in the standard testing paradigm (*e.g.,* high variability, low throughput, high cost, or confounding factors). Moreover, this integrative approach also helps to address data interpretation challenges such as human relevance, biological processes leading to apical endpoints, and the role/contribution of confounding factors (e.g., maternal systemic toxicity) in eliciting effects.

[22] https://www.regulations.gov/document/EPA-HQ-OPP-2020-0263-0054
[23] https://www.regulations.gov/document/EPA-HQ-OPP-2020-0263-0057

For malathion and malaoxon, true positive responses were limited to the rat assays tested with malathion. It is paramount to recognize that the presence of bioactivity in these assays provides evidence of potential to disrupt DNT processes, but it should not be construed as evidence that malathion is a developmental neurotoxicant *in vivo*. Although activity may be observed in the battery, it may not necessarily represent an adverse change that is typically linked to tissue-level or apical effects in a MOA/AOP. As described in the "*Toxicity Testing in the 21st Century*" report[24], to develop an AOP, not only is it necessary to establish plausible relationships among the key events, but quantitative relationships also need to be established. In other words, how much of a change in one key event is needed to result in an adverse effect at the next level of biological organization? Thus, certain exposures to a chemical may impact normal physiological responses in a way that may not necessarily be adverse. Consequently, the AOP concept requires an understanding of adaptive/homeostatic capacity of biological systems and their limits, relative to concentration and duration of exposure. At this time, OPP is taking a conservative approach by assuming that observed activity in the battery is associated with adversity.

A kinetic model is a means to assess the degree to which doses based on AChE inhibition are protective of DNT activities observed in the *in vitro* assays. A kinetic model predicts the average blood concentrations based on AChE inhibition *in vivo*, which are directly comparable with the AC_{50} values from the DNT NAM battery. The refined PBPK model-predicted concentrations associated with AChE inhibition for malathion ranged from 0.0024 to 2.1 µM for various exposure scenarios. The refined PBPK predicted average blood concentration for the steady state oral exposure scenario in rats corresponding to approximately 10% AChE inhibition was ~4800 times lower than the DNT NAM-based AC_{50} value, whereas the predicted average blood concentration for the acute oral scenario was ~300 times lower than the DNT-based AC_{50} value. The predicted average blood concentration for the dermal and inhalation exposure scenarios in rats was ~6 times lower than the DNT-based AC_{50} value. As discussed earlier, the selection of the kinetic model used for this purpose should follow a tiered approach, such that the most appropriate tool is utilized for the intended purpose. While the appropriateness of a given model is dictated by numerous factors, the highly refined PBPK model for malathion and malaoxon provides reliable predicted values and reduces uncertainty in the comparison between the blood concentrations that correspond to AChE inhibition-based doses and bioactivities observed in the DNT NAM battery. As a result, there is high confidence in the predicted concentrations reported given the robustness of the refined PBPK model.

Although there is evidence of potential DNT, results are consistent across multiple lines of evidence (*in vivo* and *in vitro*) that demonstrate AChE inhibition is protective of potential DNT effects. The DNT NAM battery which assesses hundreds of different endpoints showed no evidence of true DNT responses in either human or rat assays for malaoxon or in human assays for malathion. For the activity observed in the rat assays for malathion, the refined PBPK predicted average blood concentrations were much lower (approximately 6 – 4800 times) than the median values for all assay categories (i.e., network formation & function, NOG, and synaptogenesis). Additionally, the *in vivo* guideline DNT study, as well as the available literature study that evaluated neurobehavioral effects in laboratory animals, clearly showed that substantial AChE inhibition was occurring at dose levels where potential DNT effects were observed, and AChE inhibition would therefore be protective of these observed effects. For its part, the epidemiology evidence related to DNT outcomes across eleven

[24] http://www.nap.edu/catalog.php?record_id=11970

studies was insufficient to establish a clear associative or causal relationship between malathion/malaoxon exposure and DNT outcomes. Based on the WOE analysis presented, AChE inhibition continues to be considered the most sensitive endpoint and selecting a POD based on this effect would result in a health-protective risk assessment.

Conclusions

The DNT potential of malathion and its oxon was evaluated using chemical-specific data across multiple lines of evidence (*in vivo* toxicology studies, epidemiological studies, and the *in vitro* DNT NAM battery). The totality of the data, therefore, indicates that potential DNT effects observed for malathion and malaoxon would occur in the presence of substantial AChE inhibition, which is the basis for the current risk assessment endpoints and PODs. Based on this evaluation, AChE inhibition is considered protective of potential DNT effects from malathion/malaoxon, as supported by: (1) malathion activity in the human cell lines occurred at concentrations above where cytotoxicity occurred and there were no true positive results for malaoxon in the DNT battery in human assays, (2) lack of true positive responses for malaoxon in the DNT battery in rat assays, (3) average blood concentrations of malathion predicted at doses corresponding to approximately 10% AChE inhibition are much lower than NAM-based AC_{50} values for malathion in rat assays (~6 to 4800 fold lower), and (4) significant AChE inhibition would be occurring at doses well below those that elicited effects in the *in vivo* studies with laboratory animals, including the DNT guideline study. Epidemiology evidence related to DNT outcomes demonstrated there was insufficient evidence of a clear associative or causal relationship between malathion exposure and potential DNT outcomes.

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Appendix A

Table A.1. Efficacy cutoff methods for each DNT NAM endpoint in the ToxCast Data Analysis Pipeline (tcpl).

The tcpl level 5 methods ('mc5.mthds') for the efficacy cutoff are indicated for each multi-concentration (mc) screening assay endpoint. The efficacy cutoff methods include 3* the baseline median absolute deviation (bmad3), 20%, (pc20), bmad5, pc25, bmad2, and pc10. When multiple mc5 methods are indicated, the efficacy cutoff is defined as the maximum of all values given by the assigned level 5 methods.

Assay endpoint name	mc5.mthds
CCTE_Shafer_MEA_dev_firing_rate_mean_dn	bmad3
CCTE_Shafer_MEA_dev_burst_rate_dn	bmad3
CCTE_Shafer_MEA_dev_active_electrodes_number_dn	bmad3
CCTE_Shafer_MEA_dev_bursting_electrodes_number_dn	bmad3
CCTE_Shafer_MEA_dev_per_burst_interspike_interval_dn	bmad3
CCTE_Shafer_MEA_dev_per_burst_spike_percent_dn	bmad3
CCTE_Shafer_MEA_dev_burst_duration_mean_dn	bmad3
CCTE_Shafer_MEA_dev_interburst_interval_mean_dn	bmad3
CCTE_Shafer_MEA_dev_network_spike_number_dn	bmad3
CCTE_Shafer_MEA_dev_network_spike_peak_dn	bmad3
CCTE_Shafer_MEA_dev_spike_duration_mean_dn	bmad3
CCTE_Shafer_MEA_dev_network_spike_duration_std_dn	bmad3
CCTE_Shafer_MEA_dev_inter_network_spike_interval_mean_dn	bmad3
CCTE_Shafer_MEA_dev_per_network_spike_spike_number_mean_dn	bmad3
CCTE_Shafer_MEA_dev_per_network_spike_spike_percent_dn	bmad3
CCTE_Shafer_MEA_dev_correlation_coefficient_mean_dn	bmad3
CCTE_Shafer_MEA_dev_mutual_information_norm_dn	bmad3
CCTE_Shafer_MEA_dev_LDH_dn	bmad3
CCTE_Shafer_MEA_dev_AB_dn	bmad3
UKN5_HCS_SBAD2_neurite_outgrowth_dn	bmad3, pc20, bmad5, pc25
UKN5_HCS_SBAD2_cell_viability_dn	bmad3, pc20, bmad5, pc25
UKN2_HCS_IMR90_neural_migration_dn	bmad3, pc20, bmad5, pc25
UKN2_HCS_IMR90_cell_viability_dn	bmad3, pc20,

Assay endpoint name	mc5.mthds
	bmad5, pc25
UKN4_HCS_LUHMES_neurite_outgrowth_dn	pc25
UKN4_HCS_LUHMES_cell_viability_dn	pc25
IUF_NPC1a_proliferation_BrdU_72hr_dn	pc30, bmad2
IUF_NPC1a_proliferation_area_72hr_dn	pc30, bmad2
IUF_NPC1_viability_72hr_dn	pc30, bmad2
CCTE_Mundy_HCI_Cortical_NOG_BPCount_loss	bmad3, pc30
CCTE_Mundy_HCI_Cortical_NOG_NeuriteCount_loss	bmad3, pc30
CCTE_Mundy_HCI_Cortical_NOG_NeuriteLength_loss	bmad3, pc30
CCTE_Mundy_HCI_Cortical_NOG_NeuronCount_loss	bmad3, pc30
CCTE_Mundy_HCI_Cortical_Synap&Neur_Matur_BPCount_loss	bmad3, pc30
CCTE_Mundy_HCI_Cortical_Synap&Neur_Matur_CellBodySpotCount_loss	bmad3, pc30
CCTE_Mundy_HCI_Cortical_Synap&Neur_Matur_NeuriteCount_loss	bmad3, pc30
CCTE_Mundy_HCI_Cortical_Synap&Neur_Matur_NeuriteLength_loss	bmad3, pc30
CCTE_Mundy_HCI_Cortical_Synap&Neur_Matur_NeuriteSpotCountPerNeuriteLength_loss	bmad3, pc30
CCTE_Mundy_HCI_Cortical_Synap&Neur_Matur_NeuriteSpotCountPerNeuron_loss	bmad3, pc30
CCTE_Mundy_HCI_Cortical_Synap&Neur_Matur_NeuronCount_loss	bmad3, pc30
CCTE_Mundy_HCI_Cortical_Synap&Neur_Matur_SynapseCount_loss	bmad3, pc30
CCTE_Mundy_HCI_hN2_NOG_BPCount_loss	bmad3, pc30
CCTE_Mundy_HCI_hN2_NOG_NeuriteCount_loss	bmad3, pc30
CCTE_Mundy_HCI_hN2_NOG_NeuriteLength_loss	bmad3, pc30
CCTE_Mundy_HCI_hN2_NOG_NeuronCount_loss	bmad3, pc30
CCTE_Mundy_HCI_iCellGABA_NOG_NeuriteCount_loss	bmad3, pc30

Assay endpoint name	mc5.mthds
CCTE_Mundy_HCI_iCellGABA_NOG_NeuriteLength_loss	bmad3, pc30
CCTE_Mundy_HCI_iCellGABA_NOG_NeuronCount_loss	bmad3, pc30
CCTE_Mundy_HCI_iCellGABA_NOG_BPCount_loss	bmad3, pc30
CCTE_Mundy_HCI_hNP1_Casp3_7_gain	bmad3, pc30
CCTE_Mundy_HCI_hNP1_CellTiter_loss	bmad3, pc30
CCTE_Mundy_HCI_hNP1_Pro_MeanAvgInten_loss	bmad3, pc30
CCTE_Mundy_HCI_hNP1_Pro_ObjectCount_loss	bmad3, pc30
CCTE_Mundy_HCI_hNP1_Pro_ResponderAvgInten_loss	bmad3, pc30
IUF_NPC2a_radial_glia_migration_72hr_dn	bmad2, pc10
IUF_NPC2a_ radial_glia_migration_120hr_dn	bmad2, pc10
IUF_NPC2b_neuronal_migration_120hr_dn	pc30, bmad2
IUF_NPC2c_oligodendrocyte_migration_120hr_dn	pc30, bmad2
IUF_NPC3_neuronal_differentiation_120hr_dn	pc30, bmad2
IUF_NPC4_neurite_length_120hr_dn	pc30, bmad2
IUF_NPC4_neurite_area_120hr_dn	pc30, bmad2
IUF_NPC5_oligodendrocyte_differentiation_120hr_dn	bmad2
IUF_NPC2-5_cytotoxicity_72hr	bmad2, pc10
IUF_NPC2-5_cytotoxicity_120hr	bmad2, pc10
IUF_NPC2-5_cell_number_120hr_dn	pc30, bmad2
IUF_NPC2-5_viability_120hr_dn	pc30, bmad2
IUF_NPC1_cytotoxicity_72hr	bmad2, pc10

Table A.2. ToxCast Data Pipeline for MEA-NFA and HCI assays.

ToxCast Data Pipeline Level	MEA-NFA: Methods Applied	HCI assays: Methods Applied
mc0: pre-processed data input	Data are pre-processed to obtain AUC values by assay component	Data are raw input
mc1: mapping to well and column indexes	Auto	
mc2: transformation	No transformation	
mc3: normalization	Baseline value (bval) was calculated as the median value for the vehicle control wells (DMSO) on a by-plate basis; No positive control value was used in normalization (pval=0); the response was calculated as percent of DMSO vehicle control. The response was multiplied by -1 for the “up” endpoints such that all endpoints are curve-fit in the positive direction.	Baseline value (bval) was calculated as the median value for the vehicle control wells (DMSO) on a by-plate basis; No positive control value was used in normalization (pval=0); the response was calculated as percent of DMSO vehicle control.
mc4: BMAD calculation type for curve-fitting	An approximation of noise around the baseline signal, the baseline median absolute deviation, was calculated based on the vehicle control wells and the 2 lowest concentrations of the test wells on each plate.	An approximation of noise around the baseline signal, the baseline median absolute deviation, was calculated based on the vehicle control wells and the 2 lowest concentrations of the test wells on each plate.
mc5: Hitcall and potency determination	The cutoff for a positive response in each assay endpoint was set as 3*BMAD.	The cutoff for a positive response was the greater of 30% or 3*BMAD.
mc6: caution flags on fitting	Flags for single point hit at maximum concentration (6), flags for single point hit not at the maximum concentration screened (7), inactives with multiple median responses above baseline (8), noisy curves relative to the assay (10), actives with borderline efficacy (11), inactives with borderline efficacy (12), low concentration gain-loss curve-fits (15), possibly overfitting (16), hitcalls with less than 50% efficacy (17) were assigned to all; additionally cell viability assays	

Table A.3. Assay Activity and Endpoints Measured for Malathion in Human and Rat Neuronal Cell Lines.

Activity type	Assay short name	Species	Sample ID	AC_{50} (µM)	Average AC_{50} (µM)	Assay measure cytotoxicity (Yes/No)	Selective Activity (Yes/No)	Notes from Expert Review
Human Assays								
Apoptosis	HCI_hNP1_Casp3_7_gain	Human	EPAPLT0167G08	-	-	No	-	
	HCI_hNP1_Casp3_7_gain	Human	TT0000177D02	82.83	-	No	No	Assay was non-selective with activity only at 100 µM and above the ToxCast burst
	HCI_hNP1_CellTiter_loss	Human	EPAPLT0167G08	-	-	Yes	-	
	HCI_hNP1_CellTiter_loss	Human	TT0000177D02	41.12	-	Yes	-	Cytotoxicity assay with activity only at 100 µM and above the ToxCast burst
Proliferation	HCI_hNP1_Pro_MeanAvgInten_loss	Human	EPAPLT0167G08	-	-	No	-	
	HCI_hNP1_Pro_MeanAvgInten_loss	Human	TT0000177D02	-	-	No	-	
	HCI_hNP1_Pro_ObjectCount_loss	Human	EPAPLT0167G08	-	-	Yes	-	
	HCI_hNP1_Pro_ObjectCount_loss	Human	TT0000177D02	-	-	Yes	-	
	HCI_hNP1_Pro_ResponderAvgInten_loss	Human	EPAPLT0167G08	-	-	No	-	
	HCI_hNP1_Pro_ResponderAvgInten_loss	Human	TT0000177D02	-	-	No	-	

Activity type	Assay short name	Species	Sample ID	AC_{50} (µM)	Average AC_{50} (µM)	Assay measure cytotoxicity (Yes/No)	Selective Activity (Yes/No)	Notes from Expert Review
	IUF_NPC1_Cytotoxicity_72hr	Human	EX000567	-	-	Yes	-	
	IUF_NPC1_Viability_72hr_dn	Human	EX000567	-	-	Yes	-	
	IUF_NPC1a_Proliferation_BrdU_72hr_dn	Human	EX000567	-	-	No	-	
	IUF_NPC1a_proliferation_area_72hr_dn	Human	EX000567	-	-	No	-	
Cytotoxicity	IUF_NPC2-5_Cell_number_120hr_dn	Human	EX000567	-	-	Yes	-	
	IUF_NPC2-5_Cytotoxicity_120hr	Human	EX000567	-	-	Yes	-	
	IUF_NPC2-5_Cytotoxicity_72hr	Human	EX000567	-	-	Yes	-	
	IUF_NPC2-5_Viability_120hr_dn	Human	EX000567	-	-	Yes	-	
Migration	IUF_NPC2a_Radial_glia_migration_120hr_dn	Human	EX000567	-	-	No	-	
	IUF_NPC2a_Radial_glia_migration_72hr_dn	Human	EX000567	-	-	No	-	
	IUF_NPC2b_Neuronal_migration_120hr_dn	Human	EX000567	-	-	No	-	
	IUF_NPC2c_Oligodendrocyte_migration_120hr_dn	Human	EX000567	-	-	No	-	
Neuronal differentiation	IUF_NPC3_Neuronal_differentiation_120hr_dn	Human	EX000567	-	-	No	-	
	IUF_NPC4_Neurite_area_120hr_dn	Human	EX000567	-	-	No	-	
	IUF_NPC4_Neurite_length_120hr_dn	Human	EX000567	-	-	No	-	

Activity type	Assay short name	Species	Sample ID	AC_{50} (µM)	Average AC_{50} (µM)	Assay measure cytotoxicity (Yes/No)	Selective Activity (Yes/No)	Notes from Expert Review
Neurite Outgrowth	CCTE_Mundy_HCI_hN2_NOG_BPCount_loss	Human	TT0000177D02	34.9	-	No	No	Assay was non-selective with activity only at 100 µM and above the ToxCast burst
	CCTE_Mundy_HCI_hN2_NOG_NeuriteCount_loss	Human	TT0000177D02	39.14	-	No	No	Assay was non-selective with activity only at 100 µM and above the ToxCast burst
	CCTE_Mundy_HCI_hN2_NOG_NeuriteLength_loss	Human	TT0000177D02	38.31	-	No	No	Assay was non-selective with activity only at 100 µM and above the ToxCast burst
	CCTE_Mundy_HCI_hN2_NOG_NeuronCount_loss	Human	TT0000177D02	33.18	-	Yes	-	Cytotoxicity assay with activity only at 100 µM and above the ToxCast burst
	CCTE_Mundy_HCI_CDI_NOG_BPCount_loss	Human	EPAPLT0167G08	-	-	No	-	
	CCTE_Mundy_HCI_CDI_NOG_NeuriteCount_loss	Human	EPAPLT0167G08	16.12	-	No	-	Low confidence in the AC_{50} values from this fitting

Activity type	Assay short name	Species	Sample ID	AC_{50} (µM)	Average AC_{50} (µM)	Assay measure cytotoxicity (Yes/No)	Selective Activity (Yes/No)	Notes from Expert Review
								because it is borderline at 100 µM and overfitting.
	CCTE_Mundy_HCI_CDI_NOG_NeuriteLength_loss	Human	EPAPLT0167G08	1.37	-	No	-	Low confidence in the AC_{50} values from this fitting because it is borderline at 100 µM and overfitting.
	CCTE_Mundy_HCI_CDI_NOG_NeuronCount_loss	Human	EPAPLT0167G08	-	-	Yes	-	
	CCTE_Mundy_HCI_iCellGABA_NOG_BPCount_loss	Human	TT0000177D02	38.29	-	No	No	Assay was non-selective with activity only at 100 µM and above the ToxCast burst
	CCTE_Mundy_HCI_iCellGABA_NOG_NeuriteCount_loss	Human	TT0000177D02	42.01	-	No	No	Assay was non-selective with activity only at 100 µM and above the ToxCast burst
	CCTE_Mundy_HCI_iCellGABA_NOG_NeuriteLength_loss	Human	TT0000177D02	81.77	-	No	No	Assay was non-selective with activity only at

Activity type	Assay short name	Species	Sample ID	AC_{50} (µM)	Average AC_{50} (µM)	Assay measure cytotoxicity (Yes/No)	Selective Activity (Yes/No)	Notes from Expert Review
								100 µM and above the ToxCast burst
	CCTE_Mundy_HCI_iCellGABA_NOG_NeuronCount_loss	Human	TT0000177D02	56.07	-	Yes	No	Cytotoxicity assay with activity only at 100 µM and above the ToxCast burst
Oligodendrocyte differentiation	IUF_NPC5_Oligodendrocyte_differentiation_120hr_dn	Human	EX000567	-	-	No	-	
	IUF_NPC5_Oligodendrocyte_differentiation_120 hr_up	Human	EX000567	19.42	-	No	-	Borderline activity, high variability leading to a flat response, low confidence in the AC_{50} value, above the ToxCast burst
Rat Assays								
Neurite outgrowth	HCI_Cortical_NOG_BPCount_loss	Rat	EPAPLT0167G08	12.37	48.88	No	Yes	Activity only at the highest dose tested
	HCI_Cortical_NOG_BPCount_loss	Rat	TT0000177D02	85.39		No	No	Activity only at the highest dose tested
	HCI_Cortical_NOG_NeuriteCount_loss	Rat	EPAPLT0167G08	-	-	No	-	

Activity type	Assay short name	Species	Sample ID	AC_{50} (µM)	Average AC_{50} (µM)	Assay measure cytotoxicity (Yes/No)	Selective Activity (Yes/No)	Notes from Expert Review
	HCI_Cortical_NOG_NeuriteCount_loss	Rat	TT0000177D02	52.81	-	No	Yes	Activity only at the highest dose tested.
	HCI_Cortical_NOG_NeuriteLength_loss	Rat	EPAPLT0167G08	15.22	50.11	No	Yes	Activity only at the highest dose tested.
	HCI_Cortical_NOG_NeuriteLength_loss	Rat	TT0000177D02	84.99		No	No	Activity only at the highest dose tested.
	HCI_Cortical_NOG_NeuronCount_loss	Rat	EPAPLT0167G08	17.69	41.21	Yes	No	Activity only at the highest dose tested.
	HCI_Cortical_NOG_NeuronCount_loss	Rat	TT0000177D02	64.74		Yes	No	Activity only at the highest dose tested.
Synaptogenesis/	HCI_Cortical_Synap&Neur_Matur_BPCount_loss	Rat	EPAPLT0167G08	-	-	-	-	
	HCI_Cortical_Synap&Neur_Matur_BPCount_loss	Rat	TT0000177D02	33.20	-	No	Yes	Activity only at the highest dose tested.
	HCI_Cortical_Synap&Neur_Matur_CellBodySpotCount_loss	Rat	EPAPLT0167G08	-	-	No	-	
	HCI_Cortical_Synap&Neur_Matur_CellBodySpotCount_loss	Rat	TT0000177D02	-	-	No	-	
	HCI_Cortical_Synap&Neur_Matur_NeuriteCount_loss	Rat	EPAPLT0167G08	-	-	No	-	
	HCI_Cortical_Synap&Neur_Matur_NeuriteCount_loss	Rat	TT0000177D02	-	-	No	-	

Activity type	Assay short name	Species	Sample ID	AC_{50} (µM)	Average AC_{50} (µM)	Assay measure cytotoxicity (Yes/No)	Selective Activity (Yes/No)	Notes from Expert Review
Maturation	HCI_Cortical_Synap&Neur_Matur_NeuriteLength_loss	Rat	EPAPLT0167G08	-	-	No	-	
	HCI_Cortical_Synap&Neur_Matur_NeuriteLength_loss	Rat	TT0000177D02	35.15	-	No	Yes	Activity only at the highest dose tested.
	HCI_Cortical_Synap&Neur_Matur_NeuriteSpotCountPerNeuriteLength_loss	Rat	EPAPLT0167G08	-	-	No	-	
	HCI_Cortical_Synap&Neur_Matur_NeuriteSpotCountPerNeuriteLength_loss	Rat	TT0000177D02	-	-	No	-	
	HCI_Cortical_Synap&Neur_Matur_NeuriteSpotCountPerNeuron_loss	Rat	EPAPLT0167G08	-	-	No	-	
	HCI_Cortical_Synap&Neur_Matur_NeuriteSpotCountPerNeuron_loss	Rat	TT0000177D02	32.82	-	No	Yes	Activity only at the highest dose tested.
	HCI_Cortical_Synap&Neur_Matur_NeuronCount_loss	Rat	EPAPLT0167G08	-	-	Yes	-	
	HCI_Cortical_Synap&Neur_Matur_NeuronCount_loss	Rat	TT0000177D02	67.98	-	Yes	No	Activity only at the highest dose tested.
	HCI_Cortical_Synap&Neur_Matur_SynapseCount_loss	Rat	EPAPLT0167G08	-	-	No	-	
	HCI_Cortical_Synap&Neur_Matur_SynapseCount_loss	Rat	TT0000177D02	32.56	-	No	Yes	Activity only at the highest dose tested.
	MEA_dev_ Alamar blue _dn	Rat	EPAPLT0167G08	25.28	23.99	Yes	No	Activity at the highest

Activity type	Assay short name	Species	Sample ID	AC_{50} (µM)	Average AC_{50} (µM)	Assay measure cytotoxicity (Yes/No)	Selective Activity (Yes/No)	Notes from Expert Review
Network formation and function								concentration tested
	MEA_dev_ Alamar blue _dn	Rat	TT0000177D02	22.7		Yes	No	Clear activity above the baseline
	MEA_dev_ LDH _dn	Rat	EPAPLT0167G08	25.08	22.5	Yes	No	Activity at the highest concentration tested
	MEA_dev_ LDH _dn	Rat	TT0000177D02	19.93		Yes	No	Clear activity above the baseline
	MEA_dev_active_electrodes_number_dn	Rat	EPAPLT0167G08	7.73	11.69	No	Yes	Clear activity above the baseline
	MEA_dev_active_electrodes_number_dn	Rat	TT0000177D02	15.66		No	Yes	Clear activity above the baseline
	MEA_dev_burst_duration_mean_dn	Rat	EPAPLT0167G08	3.49	13.16	No	Yes	Variable and borderline
	MEA_dev_burst_duration_mean_dn	Rat	TT0000177D02	22.84		No	No	Borderline, some variability
	MEA_dev_burst_rate_dn	Rat	EPAPLT0167G08	2.93	11.6	No	Yes	Borderline
	MEA_dev_burst_rate_dn	Rat	TT0000177D02	20.27		No	No	Borderline
	MEA_dev_bursting_electrodes_number_dn	Rat	EPAPLT0167G08	5.07	8.45	No	Yes	Clear activity above the baseline

Activity type	Assay short name	Species	Sample ID	AC_{50} (µM)	Average AC_{50} (µM)	Assay measure cytotoxicity (Yes/No)	Selective Activity (Yes/No)	Notes from Expert Review
	MEA_dev_bursting_electrodes_number_dn	Rat	TT0000177D02	11.83		No	Yes	Clear activity above the baseline
	MEA_dev_correlation_coefficient_mean_dn	Rat	EPAPLT0167G08	12.71	11.48	No	Yes	Variable with activity only at the highest concentration
	MEA_dev_correlation_coefficient_mean_dn	Rat	TT0000177D02	10.26		No	Yes	Clear activity above the baseline
	MEA_dev_firing_rate_mean_dn	Rat	EPAPLT0167G08	0.80	9.55	No	Yes	Borderline
	MEA_dev_firing_rate_mean_dn	Rat	TT0000177D02	18.3		No	Yes	Borderline
	MEA_dev_inter_network_spike_interval_mean_dn	Rat	EPAPLT0167G08	-	-	No	-	
	MEA_dev_inter_network_spike_interval_mean_dn	Rat	TT0000177D02	10.33	-	No	Yes	Borderline
	MEA_dev_interburst_interval_mean_dn	Rat	EPAPLT0167G08	11.37	12.57	No	Yes	Borderline
	MEA_dev_interburst_interval_mean_dn	Rat	TT0000177D02	13.74		No	Yes	Borderline
	MEA_dev_mutual_information_norm_dn	Rat	EPAPLT0167G08	3.92	5.63	No	Yes	Borderline
	MEA_dev_mutual_information_norm_dn	Rat	TT0000177D02	7.33		No	Yes	Borderline
	MEA_dev_network_spike_duration_std_dn	Rat	EPAPLT0167G08	8.47	10.08	No	Yes	Borderline
	MEA_dev_network_spike_duration_std_dn	Rat	TT0000177D02	11.69		No	Yes	Borderline

Activity type	Assay short name	Species	Sample ID	AC_{50} (µM)	Average AC_{50} (µM)	Assay measure cytotoxicity (Yes/No)	Selective Activity (Yes/No)	Notes from Expert Review
	MEA_dev_network_spike_number_dn	Rat	EPAPLT0167G08	11.24	11.66	No	Yes	Borderline
	MEA_dev_network_spike_number_dn	Rat	TT0000177D02	12.08		No	Yes	Borderline
	MEA_dev_network_spike_peak_dn	Rat	EPAPLT0167G08	9.13	10.68	No	Yes	Clear activity above the baseline
	MEA_dev_network_spike_peak_dn	Rat	TT0000177D02	12.23		No	Yes	Clear activity above the baseline
	MEA_dev_per_burst_interspike_interval_dn	Rat	EPAPLT0167G08	4.16	15.24	No	Yes	Borderline
	MEA_dev_per_burst_interspike_interval_dn	Rat	TT0000177D02	26.33		No	No	Borderline
	MEA_dev_per_burst_spike_percent_dn	Rat	EPAPLT0167G08	9.50	13.7	No	Yes	Clear activity above the baseline
	MEA_dev_per_burst_spike_percent_dn	Rat	TT0000177D02	17.9		No	Yes	Clear activity above the baseline
	MEA_dev_per_network_spike_spike_number_mean_dn	Rat	EPAPLT0167G08	8.90	10.7	No	Yes	Activity at the highest concentration tested, otherwise borderline
	MEA_dev_per_network_spike_spike_number_mean_dn	Rat	TT0000177D02	12.5		No	Yes	Clear activity above the baseline

Activity type	Assay short name	Species	Sample ID	AC_{50} (µM)	Average AC_{50} (µM)	Assay measure cytotoxicity (Yes/No)	Selective Activity (Yes/No)	Notes from Expert Review
	MEA_dev_per_network_spike_spike_percent_dn	Rat	EPAPLT0167G08	-	-	No	-	
	MEA_dev_per_network_spike_spike_percent_dn	Rat	TT0000177D02	11.31	-	No	Yes	Clear activity above the baseline
	MEA_dev_spike_duration_mean_dn	Rat	EPAPLT0167G08	6.44	9.35	No	Yes	Clear activity above the baseline
	MEA_dev_spike_duration_mean_dn	Rat	TT0000177D02	12.26		No	No	Clear activity above the baseline

Key:

- The assay name is comprised of the name of the assay, type of cell line used and assay endpoint.
- MEA = microelectrode array network formation assay; HCI = high-content imaging; hNP1= human neural progenitor cell line; NPC1-4 = human primary neuroprogenitor cells (Lonza) in neurosphere cultures & NPC5 = human glial cells in neurosphere cultures developed at Leibniz Institute for Environmental Medicine (IUF). UKN2 = human neural crest from h9 embryonic stem cells; UKN4 = Lund human mesencephalic human embryonic neuronal precursor (LUHMES) cells; UKN5 = human peripheral nervous system cells (immature dorsal root ganglion) cells from h9 embryonic cells, developed at University of Konstanz (UKN).
- Assay with no activity is represented by "-" in the AC_{50} column.
- Average AC_{50} calculated when positive results were obtained from both samples for a single DNT endpoint.
- Assay selectivity: Selectivity scores of > 0.3 likely indicate some selective activity of the chemical in the assay, i.e., the lowest concentration-related effects occurred at lower concentrations than cytotoxicity.

Table A.4 Assay Activity and Endpoints Measured for Malaoxon in Human and Rat Neuronal Cell Lines.

Activity type	Assay short name	Species	Sample ID	AC_{50} (µM)	Assay measured cytotoxicity (Yes/No)	Selective Activity (Yes/No)	Notes from Expert Review
Human Assays							
Apoptosis	HCI_hNP1_Casp3_7_gain	Human	EX000566	-	No	-	
	HCI_hNP1_CellTiter_loss	Human	EX000566	-	Yes	-	
Proliferation	HCI_hNP1_Pro_MeanAvgInten_loss	Human	EX000566	-	No	-	
	HCI_hNP1_Pro_ObjectCount_loss	Human	EX000566	-	Yes	-	
	HCI_hNP1_Pro_ResponderAvgInten_loss	Human	EX000566	-	No	-	
	IUF_NPC1_Cytotoxicity_72hr	Human	EX000566	-	Yes	-	
	IUF_NPC1_Viability_72hr_dn	Human	EX000566	0.85	Yes	-	No activity in corresponding cytotoxicity assay; low confidence due to variability/noisy data
	IUF_NPC1a_Proliferation_BrdU_72hr_dn	Human	EX000566	4.58	No	-	No activity in corresponding cytotoxicity assay; low confidence due to variability/noisy data
	IUF_NPC1a_proliferation_area_72hr_dn	Human	EX000566	-	No	-	

Activity type	Assay short name	Species	Sample ID	AC_{50} (µM)	Assay measured cytotoxicity (Yes/No)	Selective Activity (Yes/No)	Notes from Expert Review
Cytotoxicity	IUF_NPC2-5_Cell_number_120hr_dn	Human	EX000566	-	Yes	-	
	IUF_NPC2-5_Cytotoxicity_120hr	Human	EX000566	-	Yes	-	
	IUF_NPC2-5_Cytotoxicity_72hr	Human	EX000566	-	Yes	-	
	IUF_NPC2-5_Viability_120hr_dn	Human	EX000566	-	Yes	-	
Neurite outgrowth (CDI)	CCTE_Mundy_HCI_iCellGABA_NOG_BPCount_loss	Human	EX000566	-	No	-	
	CCTE_Mundy_HCI_iCellGABA_NOG_NeuriteCount_loss	Human	EX000566	-	No	-	
	CCTE_Mundy_HCI_iCellGABA_NOG_NeuriteLength_loss	Human	EX000566	-	No	-	
	CCTE_Mundy_HCI_iCellGABA_NOG_NeuronCount_loss	Human	EX000566	-	Yes	-	
Migration	IUF_NPC2a_Radial_glia_migration_120hr_dn	Human	EX000566	-	No	-	
	IUF_NPC2a_Radial_glia_migration_72hr_dn	Human	EX000566	-	No	-	
	IUF_NPC2b_Neuronal_migration_120hr_dn	Human	EX000566	-	No	-	
	IUF_NPC2c_Oligodendrocyte_migration_120hr_dn	Human	EX000566	-	No	-	
Neuronal differentiation	IUF_NPC3_Neuronal_differentiation_120hr_dn	Human	EX000566	-	No	-	
Neurite outgrowth	IUF_NPC4_Neurite_area_120hr_dn	Human	EX000566	-	No	-	

Activity type	Assay short name	Species	Sample ID	AC_{50} (µM)	Assay measured cytotoxicity (Yes/No)	Selective Activity (Yes/No)	Notes from Expert Review
	IUF_NPC4_Neurite_length_120hr_dn	Human	EX000566	0.03	No	-	No activity in corresponding cytotoxicity assay; flat response; low confidence due to model overfitting
Oligodendrocyte differentiation	IUF_NPC5_Oligodendrocyte_differentiation_120hr_dn	Human	EX000566	-	No	-	
Rat Assays							
Neurite outgrowth initiation	HCI_Cortical_NOG_BPCount_loss	Rat	TT0000177B03	-	No	-	
	HCI_Cortical_NOG_NeuriteCount_loss	Rat	TT0000177B03	-	No	-	
	HCI_Cortical_NOG_NeuriteLength_loss	Rat	TT0000177B03	-	No	-	
	HCI_Cortical_NOG_NeuronCount_loss	Rat	TT0000177B03	-	Yes	-	
Synaptogenesis/ Maturation	HCI_Cortical_Synap&Neur_Matur_BPCount_loss	Rat	TT0000177B03	-	No	-	
	HCI_Cortical_Synap&Neur_Matur_CellBodySpotCount_loss	Rat	TT0000177B03	-	No	-	
	HCI_Cortical_Synap&Neur_Matur_NeuriteCount_loss	Rat	TT0000177B03	-	No		
	HCI_Cortical_Synap&Neur_Matur_NeuriteLength_loss	Rat	TT0000177B03	-	No		
	HCI_Cortical_Synap&Neur_Matur_NeuriteSpotCountPerNeuriteLength_loss	Rat	TT0000177B03	-	No		

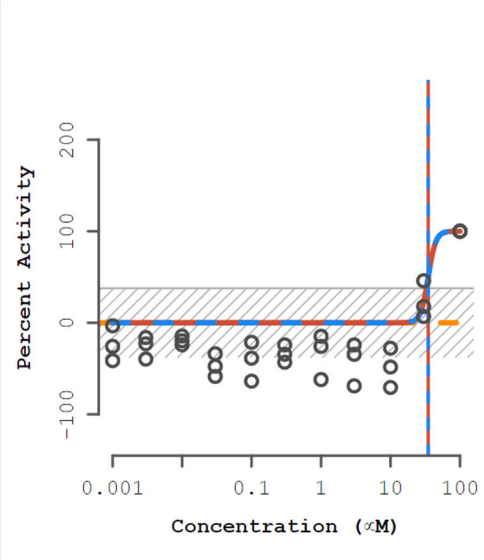
Activity type	Assay short name	Species	Sample ID	AC_{50} (µM)	Assay measured cytotoxicity (Yes/No)	Selective Activity (Yes/No)	Notes from Expert Review
	HCI_Cortical_Synap&Neur_Matur_NeuriteSpotCountPerNeuron_loss	Rat	TT0000177B03	-	No		
	HCI_Cortical_Synap&Neur_Matur_NeuronCount_loss	Rat	TT0000177B03	-	Yes		
	HCI_Cortical_Synap&Neur_Matur_SynapseCount_loss	Rat	TT0000177B03	-	No		
Network formation and function	MEA_dev_ Alamar blue _dn	Rat	TT0000177B03	-	Yes		
	MEA_dev_ LDH _dn	Rat	TT0000177B03	-	Yes		
	MEA_dev_active_electrodes_number_dn	Rat	TT0000177B03	-	No		
	MEA_dev_burst_duration_mean_dn	Rat	TT0000177B03	-	No		
	MEA_dev_burst_rate_dn	Rat	TT0000177B03	-	No		
	MEA_dev_bursting_electrodes_number_dn	Rat	TT0000177B03	-	No		
	MEA_dev_correlation_coefficient_mean_dn	Rat	TT0000177B03	-	No		
	MEA_dev_firing_rate_mean_dn	Rat	TT0000177B03	-	No		
	MEA_dev_inter_network_spike_interval_mean_dn	Rat	TT0000177B03	-	No		
	MEA_dev_interburst_interval_mean_dn	Rat	TT0000177B03	-	No		
	MEA_dev_mutual_information_norm_dn	Rat	TT0000177B03	-	No		
	MEA_dev_network_spike_duration_std_dn	Rat	TT0000177B03	-	No		
	MEA_dev_network_spike_number_dn	Rat	TT0000177B03	-	No		

Activity type	Assay short name	Species	Sample ID	AC_{50} (µM)	Assay measured cytotoxicity (Yes/No)	Selective Activity (Yes/No)	Notes from Expert Review
	MEA_dev_network_spike_peak_dn	Rat	TT0000177B03	-	No		
	MEA_dev_per_burst_interspike_interval_dn	Rat	TT0000177B03	-	No		
	MEA_dev_per_burst_spike_percent_dn	Rat	TT0000177B03	-	No		
	MEA_dev_per_network_spike_spike_number_mean_dn	Rat	TT0000177B03	-	No		
	MEA_dev_per_network_spike_spike_percent_dn	Rat	TT0000177B03	-	No		
	MEA_dev_spike_duration_mean_dn	Rat	TT0000177B03	-	No		

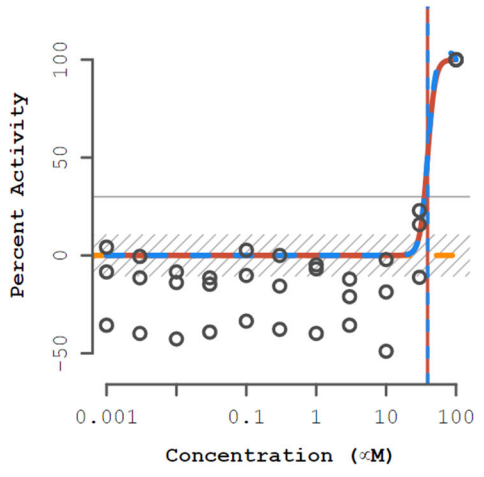
Key:

- The assay name is comprised of the name of the assay, type of cell line used and assay endpoint.
- MEA = microelectrode array network formation assay; HCI = high-content imaging; hNP1= human neural progenitor cell line; NPC1-4 = human primary neuroprogenitor cells (Lonza) in neurosphere cultures & NPC5 = human glial cells in neurosphere cultures developed at Leibniz Institute for Environmental Medicine (IUF). UKN2 = human neural crest from h9 embryonic stem cells; UKN4 = Lund human mesencephalic human embryonic neuronal precursor (LUHMES) cells; UKN5 = human peripheral nervous system cells (immature dorsal root ganglion) cells from h9 embryonic cells, developed at University of Konstanz (UKN).
- Assay with no activity is represented by "-" in the AC_{50} column.
- Average AC_{50} calculated when positive results were obtained from both samples for a single DNT endpoint.

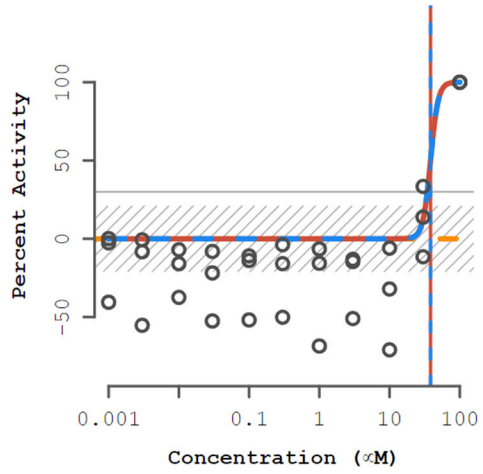
Appendix B.1 Concentration Response Curve for Malathion in Human Cell Lines.



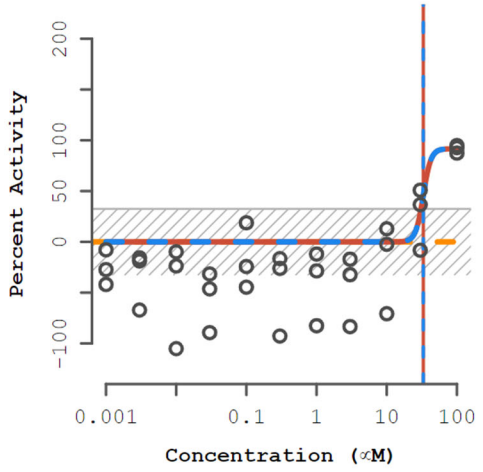

```
ASSAY:    AEID2789 (CCTE_Mundy_HCI_hN2_NOG_BPCount_loss)

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:     43468206

HILL MODEL (in red):
       tp        ga        gw
val:   100       1.54      8
sd:    16.4      0.338     40.4

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   100       1.54      8         2.51      7.26
sd:    NaN       NaN       NaN       NaN       NaN

       CNST         HILL         GNLS
AIC:   353.27       341.89       345.89
PROB:  0            0.88         0.12
RMSE:  47.44        35.94        35.94

MAX_MEAN: 100        MAX_MED: 100        BMAD: 12.6

COFF: 37.7    HIT-CALL: 1     FITC: 41    ACTP: 1
```



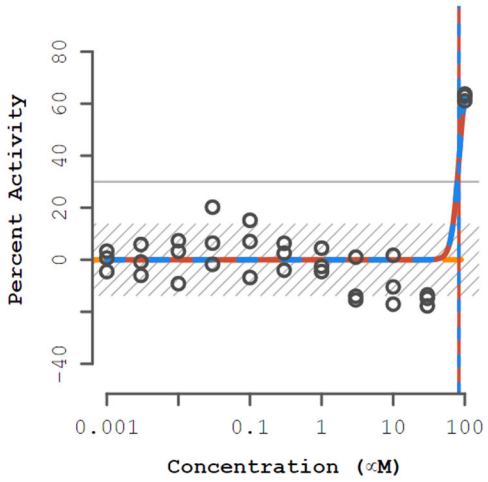

```
ASSAY:    AEID2790 (CCTE_Mundy_HCI_hN2_NOG_NeuriteCount_

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:     43468098

HILL MODEL (in red):
       tp        ga        gw
val:   100       1.59      8
sd:    11.4      1.42      97.7

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   104       1.6       7.97      2.1       14.5
sd:    247       1.41      94.9      0.0677    258

       CNST         HILL         GNLS
AIC:   330.76       308.71       312.71
PROB:  0            0.88         0.12
RMSE:  37.79        22.62        22.62

MAX_MEAN: 100        MAX_MED: 100        BMAD: 3.53

COFF: 30      HIT-CALL: 1     FITC: 41    ACTP: 1
```



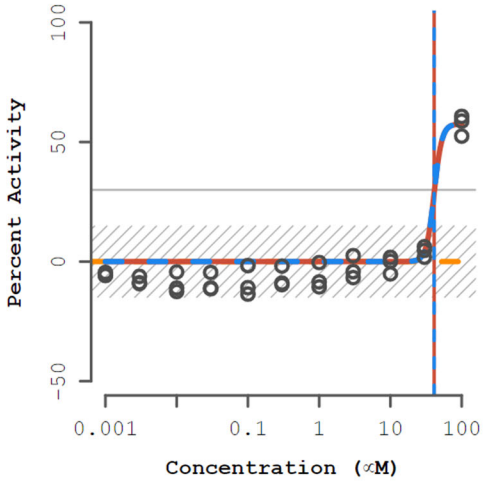

```
ASSAY:   AEID2791 (CCTE_Mundy_HCI_hN2_NOG_NeuriteLength_

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    43468025

HILL MODEL (in red):
      tp       ga       gw
val:  100      1.58     8
sd:   12.7     0.991    73.9

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  100      1.58     8        3.3      4.2
sd:   13.5     1.06     78.8     3120     10000

      CNST       HILL       GNLS
AIC:  343.07     326.79     330.79
PROB: 0          0.88       0.12
RMSE: 43.03      30.49      30.49

MAX_MEAN: 100      MAX_MED: 100      BMAD: 6.96

COFF: 30    HIT-CALL: 1    FITC: 41    ACTP: 1
```



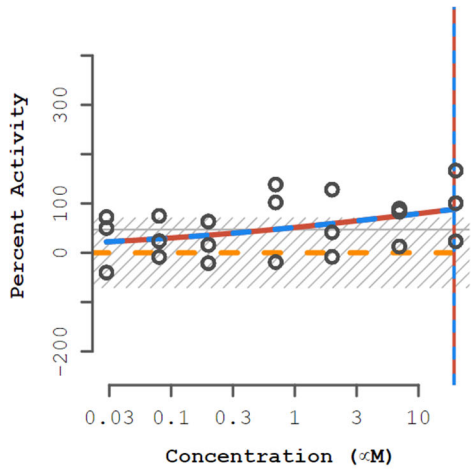

```
ASSAY:   AEID2792 (CCTE_Mundy_HCI_hN2_NOG_NeuronCount_l

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    43467943

HILL MODEL (in red):
      tp       ga       gw
val:  91.6     1.52     8
sd:   18.2     0.355    63.8

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  91.7     1.52     8        2.74     7.16
sd:   NaN      NaN      NaN      NaN      NaN

      CNST       HILL       GNLS
AIC:  360.63     352.73     356.73
PROB: 0.02       0.87       0.12
RMSE: 53.23      44.79      44.79

MAX_MEAN: 91.6      MAX_MED: 92.7      BMAD: 10.8

COFF: 32.5    HIT-CALL: 1    FITC: 41    ACTP: 0.98
```



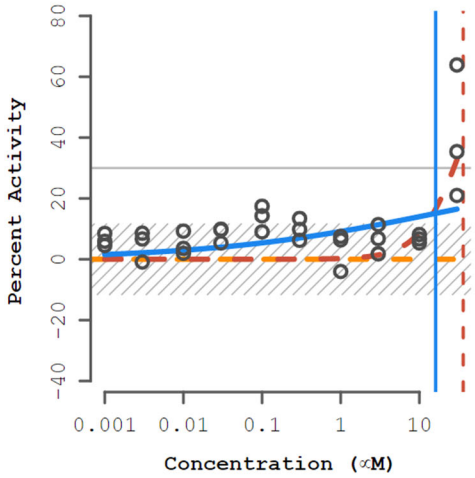

```
ASSAY:   AEID2793 (CCTE_Mundy_HCI_hNP1_Casp3_7_gain)

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    43467691

HILL MODEL (in red):
      tp       ga       gw
val:  76.4     1.92     8
sd:   46.9     0.119    9.68

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  76.4     1.92     8        3.94     16.3
sd:   NA       NA       NA       NA       NA

      CNST       HILL       GNLS
AIC:  281.14     248.38     252.38
PROB: 0          0.88       0.12
RMSE: 20.92      9.04       9.04

MAX_MEAN: 62.6      MAX_MED: 62.9      BMAD: 4.58

COFF: 30     HIT-CALL: 1    FITC: 42   ACTP: 1
```



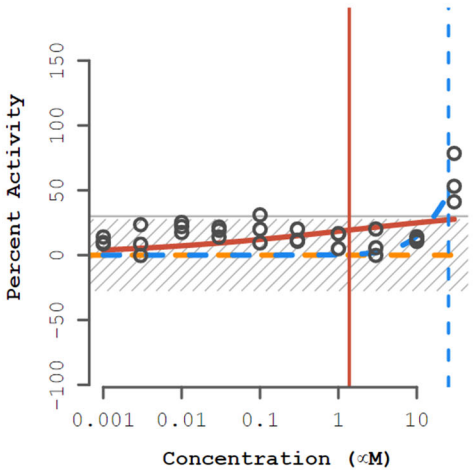

```
ASSAY:   AEID2794 (CCTE_Mundy_HCI_hNP1_CellTiter_loss)

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    43469035

HILL MODEL (in red):
      tp       ga       gw
val:  57.5     1.61     8
sd:   NaN      NaN      NaN

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  57.5     1.61     8        3.92     4.56
sd:   NaN      NaN      NaN      NaN      NaN

      CNST       HILL       GNLS
AIC:  270.55     235.22     239.22
PROB: 0          0.88       0.12
RMSE: 18.73      7.12       7.12

MAX_MEAN: 57.3      MAX_MED: 58.8      BMAD: 4.96

COFF: 30     HIT-CALL: 1    FITC: 41   ACTP: 1
```




```
ASSAY:   AEID2951 (IUF_NPC5_oligodendrocyte_differentia

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): EX000567
M4ID:    43532951

HILL MODEL (in red):
      tp       ga       gw
val:  176      1.29     0.3
sd:   689      10.7     0.535

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  176      1.29     0.3      1.87     17.8
sd:   686      10.7     0.533    4590     142000

      CNST       HILL       GNLS
AIC:  1217.77    1149.14    1153.14
PROB: 0          0.88       0.12
RMSE: 76.21      51.18      51.18

MAX_MEAN: 96.9      MAX_MED: 102       BMAD: 23.6

COFF: 47.1   HIT-CALL: 1    FITC: 42   ACTP: 1
```

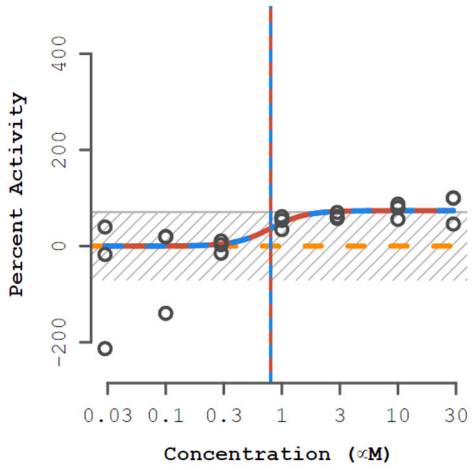



```
ASSAY:   AEID3068 (CCTE_Mundy_HCI_CDI_NOG_NeuriteCount_

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:    43471386

HILL MODEL (in red):
      tp       ga       gw
val:  76.6     1.56     1.6
sd:   108      0.75     1.38

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  30.2     1.21     0.3      2.22     10.4
sd:   NaN      NaN      NaN      NaN      NaN

      CNST       HILL       GNLS
AIC:  242.6      229.12     227.03
PROB: 0          0.26       0.74
RMSE: 16         9.73       10.99

MAX_MEAN: 40.1      MAX_MED: 35.4      BMAD: 3.87

COFF: 30     HIT-CALL: 1    FITC: 47   ACTP: 1
```

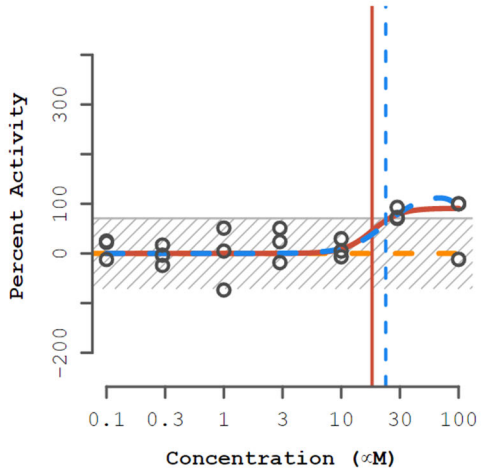



```
ASSAY:    AEID3069 (CCTE_Mundy_HCI_CDI_NOG_NeuriteLength

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:     43471543

HILL MODEL (in red):
       tp        ga        gw
val:   38.6      0.139     0.3
sd:    NaN       NaN       NaN

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   94.1      1.4       1.8       3.48      18
sd:    NA        NA        NA        NA        NA

       CNST         HILL         GNLS
AIC:   276.36       254.05       265.79
PROB:  0            1            0
RMSE:  24.28        14.97        15.52

MAX_MEAN: 57.5       MAX_MED: 53.1       BMAD: 9.14

COFF: 30      HIT-CALL: 1     FITC: 42    ACTP: 1
```

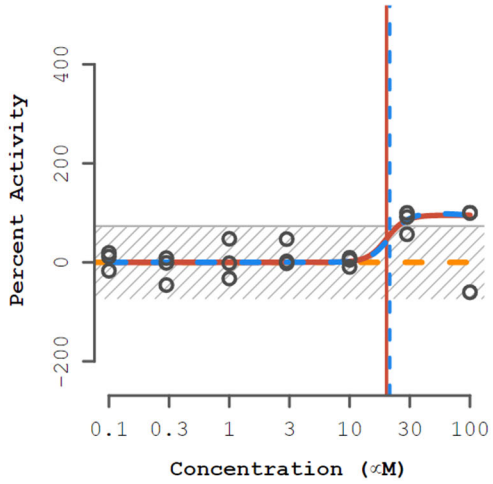
Appendix B.2. Concentration Response Curve for Malathion in Rat Cell Lines.



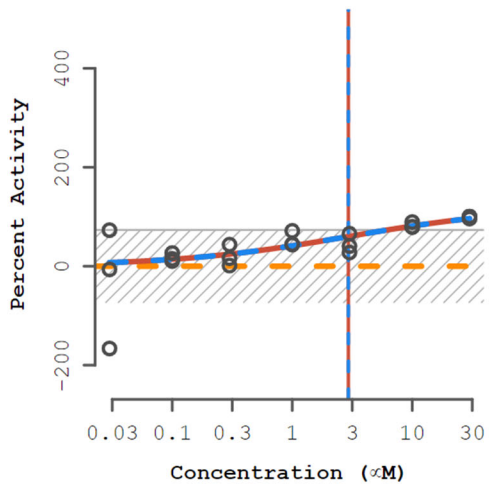

```
ASSAY:    AEID2494 (CCTE_Shafer_MEA_dev_firing_rate_mean

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:     42616995

HILL MODEL (in red):
       tp        ga        gw
val:   73.7      -0.0959   2.85
sd:    11.2      0.126     4.31

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   73.7      -0.0959   2.85      3.29      7.12
sd:    NaN       NaN       NaN       NaN       NaN

       CNST         HILL         GNLS
AIC:   244.54       221.37       225.37
PROB:  0            0.88         0.12
RMSE:  77.76        58.37        58.37

MAX_MEAN: 81.9       MAX_MED: 100        BMAD: 23.6

COFF: 70.7    HIT-CALL: 1     FITC: 37    ACTP: 1
```



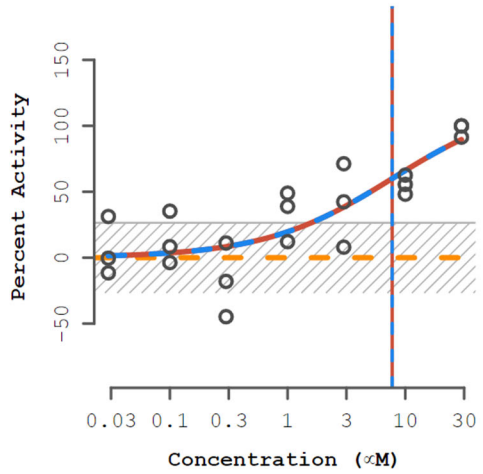

```
ASSAY:   AEID2494 (CCTE_Shafer_MEA_dev_firing_rate_mean

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    42617265

HILL MODEL (in red):
      tp       ga       gw
val:  91       1.26     3.66
sd:   17.3     0.155    2.3

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  120      1.38     2.8      2.07     7.32
sd:   1480     4.72     22.7     0.897    210

      CNST       HILL       GNLS
AIC:  227.25     213.27     217.24
PROB: 0          0.88       0.12
RMSE: 50.53      34.75      34.72

MAX_MEAN: 78.6      MAX_MED: 100       BMAD: 23.6

COFF: 70.7   HIT-CALL: 1    FITC: 41   ACTP: 1
```



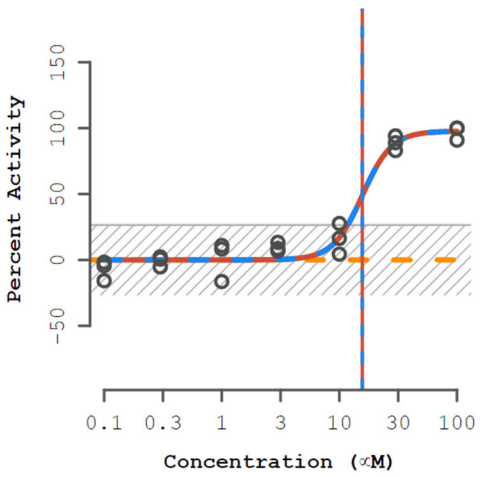

```
ASSAY:   AEID2496 (CCTE_Shafer_MEA_dev_burst_rate_dn)

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    42618102

HILL MODEL (in red):
      tp       ga       gw
val:  95.2     1.31     5.52
sd:   13.2     0.195    5.67

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  103      1.33     5.17     2.85     1.31
sd:   1180     3.38     39.8     45.9     78

      CNST       HILL       GNLS
AIC:  226.72     210.47     214.47
PROB: 0          0.88       0.12
RMSE: 50.68      40.25      40.25

MAX_MEAN: 82.7      MAX_MED: 100       BMAD: 24.4

COFF: 73.2   HIT-CALL: 1    FITC: 41   ACTP: 1
```



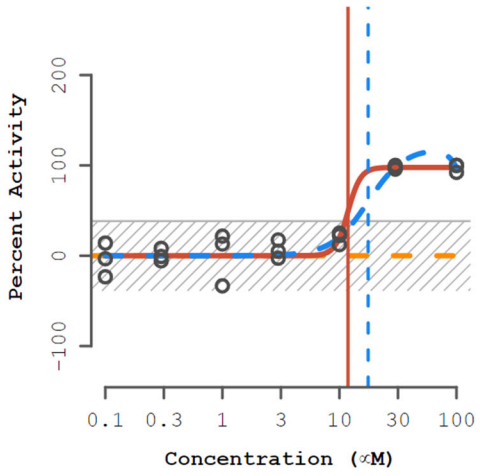

```
ASSAY:   AEID2496 (CCTE_Shafer_MEA_dev_burst_rate_dn)

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:    42618240

HILL MODEL (in red):
      tp       ga       gw
val:  120      0.468    0.601
sd:   29.9     0.468    0.189

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  120      0.468    0.601    2.72     17.8
sd:   NA       NA       NA       NA       NA

      CNST       HILL       GNLS
AIC:  241.65     205.61     209.61
PROB: 0          0.88       0.12
RMSE: 69.61      42.68      42.68

MAX_MEAN: 98.8      MAX_MED: 100       BMAD: 24.4

COFF: 73.2   HIT-CALL: 1    FITC: 42   ACTP: 1
```



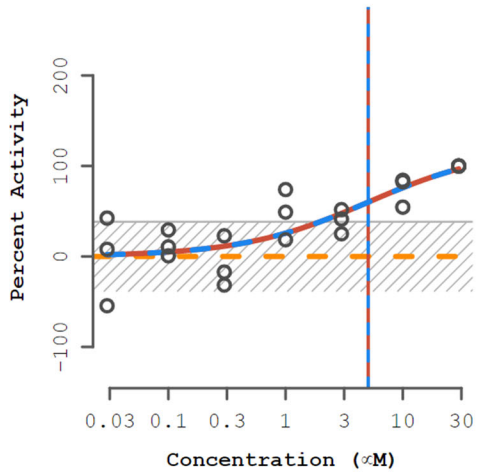

```
ASSAY:   AEID2498 (CCTE_Shafer_MEA_dev_active_electrode:

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:    42618875

HILL MODEL (in red):
      tp       ga       gw
val:  120      0.889    0.798
sd:   39       0.442    0.271

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  120      0.889    0.798    2.91     5.98
sd:   39       0.442    0.272    1640     6830

      CNST       HILL       GNLS
AIC:  227.93     197.09     201.09
PROB: 0          0.88       0.12
RMSE: 50.49      21.42      21.42

MAX_MEAN: 97.1      MAX_MED: 100       BMAD: 8.82

COFF: 26.5   HIT-CALL: 1    FITC: 42   ACTP: 1
```



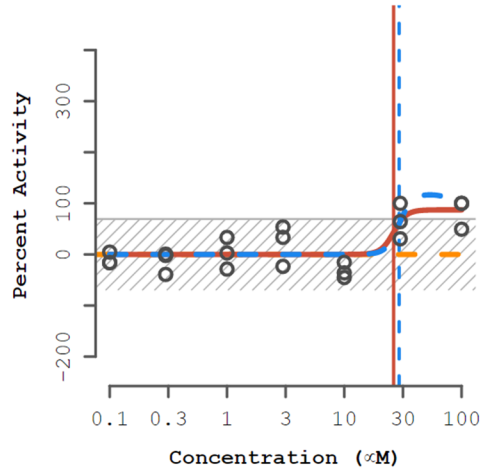

```
ASSAY:   AEID2498 (CCTE_Shafer_MEA_dev_active_electrode:

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    42618964

HILL MODEL (in red):
      tp       ga       gw
val:  97.7     1.2      3.47
sd:   4.28     0.0471   0.748

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  97.7     1.2      3.47     2.81     12.5
sd:   NaN      NaN      NaN      NaN      NaN

      CNST       HILL       GNLS
AIC:  225.01     158.13     162.13
PROB: 0          0.88       0.12
RMSE: 50.72      8.25       8.25

MAX_MEAN: 97       MAX_MED: 100       BMAD: 8.82

COFF: 26.5   HIT-CALL: 1    FITC: 41   ACTP: 1
```



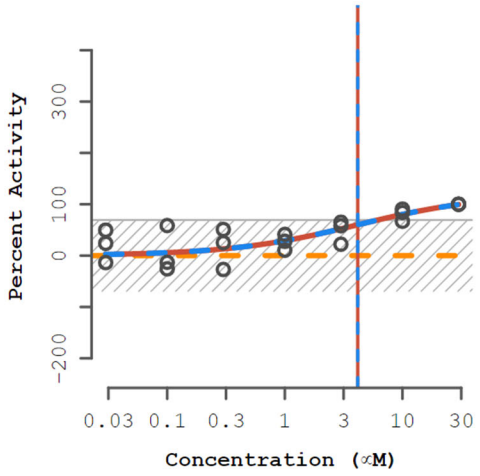

```
ASSAY:   AEID2500 (CCTE_Shafer_MEA_dev_bursting_electro

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    42619999

HILL MODEL (in red):
      tp       ga       gw
val:  97.8     1.07     8
sd:   NaN      NaN      NaN

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  120      1.25     2.78     2.1      6.28
sd:   92       0.397    3        0.0792   17.8

      CNST       HILL       GNLS
AIC:  228.65     170.13     173.83
PROB: 0          0.86       0.14
RMSE: 54.12      12.1       12.04

MAX_MEAN: 97.9     MAX_MED: 100       BMAD: 12.8

COFF: 38.3   HIT-CALL: 1    FITC: 41   ACTP: 1
```



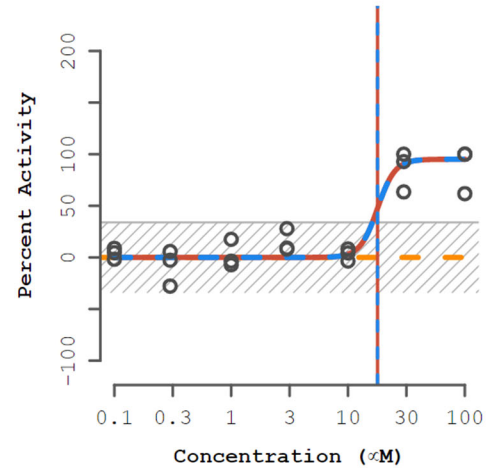

```
ASSAY:    AEID2500 (CCTE_Shafer_MEA_dev_bursting_electro

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:     42620059

HILL MODEL (in red):
       tp        ga        gw
val:   120       0.705     0.801
sd:    33.5      0.389     0.331

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   120       0.705     0.801     2.94      5.33
sd:    33.5      0.389     0.331     1040      3820

       CNST         HILL         GNLS
AIC:   233.68       201.42       205.42
PROB:  0            0.88         0.12
RMSE:  56.78        24.28        24.28

MAX_MEAN: 100       MAX_MED: 100       BMAD: 12.8

COFF: 38.3    HIT-CALL: 1     FITC: 42    ACTP: 1
```



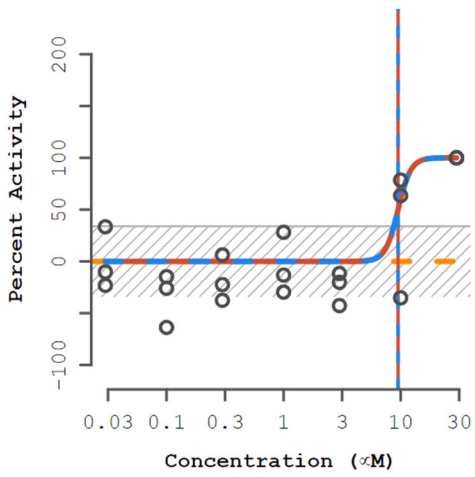

```
ASSAY:    AEID2502 (CCTE_Shafer_MEA_dev_per_burst_inters

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:     42620824

HILL MODEL (in red):
       tp        ga        gw
val:   87.3      1.42      8
sd:    16.6      0.113     13.2

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   119       1.47      7.96      2.08      5.57
sd:    874       0.858     19.9      0.917     116

       CNST         HILL         GNLS
AIC:   225.48       210.11       214.11
PROB:  0            0.88         0.12
RMSE:  48.61        27.86        27.86

MAX_MEAN: 83.1      MAX_MED: 100       BMAD: 23.1

COFF: 69.3    HIT-CALL: 1     FITC: 41    ACTP: 1
```



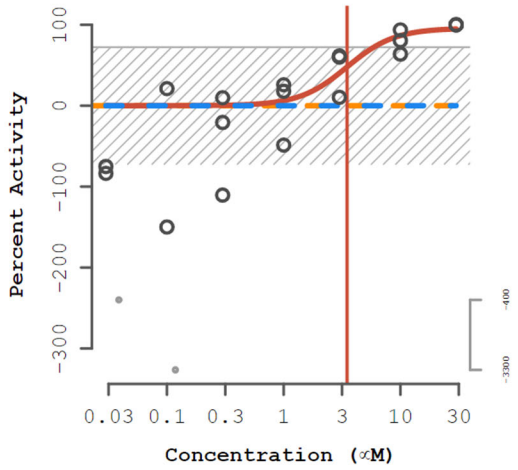

```
ASSAY:    AEID2502 (CCTE_Shafer_MEA_dev_per_burst_interspi

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:     42621039

HILL MODEL (in red):
       tp        ga        gw
val:   120       0.62      0.8
sd:    40.4      0.475     0.441

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   120       0.62      0.8       2.42      7
sd:    40.4      0.475     0.441     516       3810

       CNST         HILL         GNLS
AIC:   235.19       202.13       206.13
PROB:  0            0.88         0.12
RMSE:  58.48        24.07        24.07

MAX_MEAN: 100        MAX_MED: 100        BMAD: 23.1

COFF: 69.3    HIT-CALL: 1     FITC: 42    ACTP: 1
```



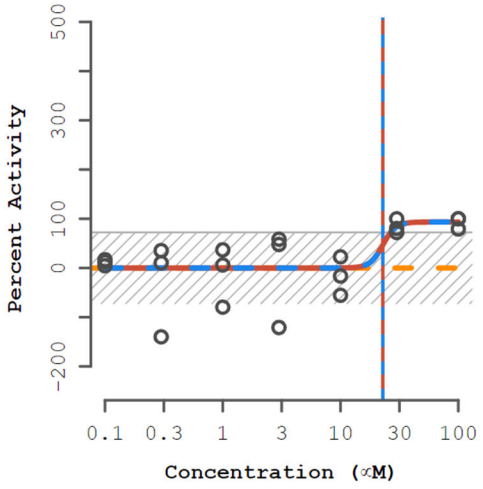

```
ASSAY:    AEID2504 (CCTE_Shafer_MEA_dev_per_burst_spike_pi

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:     42621588

HILL MODEL (in red):
       tp        ga        gw
val:   95.1      1.25      5.8
sd:    7.66      0.119     2.81

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   95.1      1.25      5.8       3.13      14.1
sd:    NA        NA        NA        NA        NA

       CNST         HILL         GNLS
AIC:   222.3        177.94       181.94
PROB:  0            0.88         0.12
RMSE:  48.19        14.25        14.25

MAX_MEAN: 87.2       MAX_MED: 100        BMAD: 11.3

COFF: 33.9    HIT-CALL: 1     FITC: 41    ACTP: 1
```



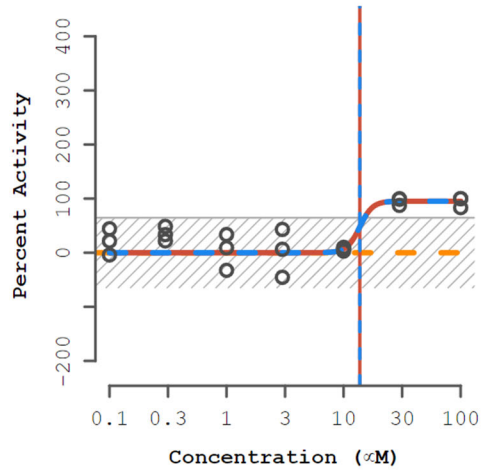

```
ASSAY:    AEID2504 (CCTE_Shafer_MEA_dev_per_burst_spike_

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:     42621915

HILL MODEL (in red):
       tp        ga        gw
val:   100       0.978     8
sd:    12.6      0.0808    25.2

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   100       0.978     8         2.79      17.9
sd:    NA        NA        NA        NA        NA

       CNST         HILL         GNLS
AIC:   227.73       212.36       216.36
PROB:  0            0.88         0.12
RMSE:  50.85        32.58        32.57

MAX_MEAN: 100        MAX_MED: 100        BMAD: 11.3

COFF: 33.9    HIT-CALL: 1    FITC: 41    ACTP: 1
```



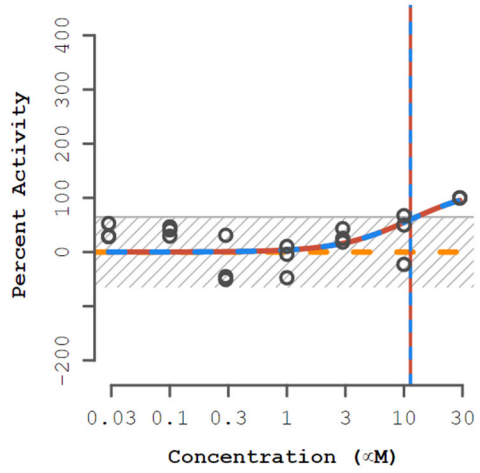

```
ASSAY:    AEID2506 (CCTE_Shafer_MEA_dev_burst_duration_m

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:     42622583  BRK

HILL MODEL (in red):
       tp        ga        gw
val:   95.8      0.543     2.13
sd:    37.3      0.447     3.22

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   101       -2.5      0.535     -2.23     10.5
sd:    NaN       NaN       NaN       NaN       NaN

       CNST         HILL         GNLS
AIC:   284.85       280.65       294.85
PROB:  0.11         0.89         0
RMSE:  737.34       735.6        737.34

MAX_MEAN: 100        MAX_MED: 100        BMAD: 24.1

COFF: 72.3    HIT-CALL: 1    FITC: 41    ACTP: 0.89
```



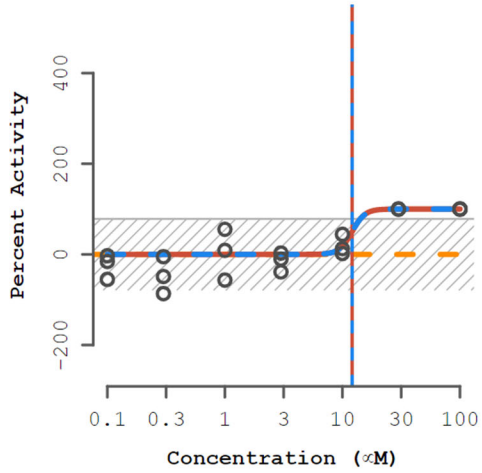

```
ASSAY:    AEID2506 (CCTE_Shafer_MEA_dev_burst_duration_m

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:     42622777

HILL MODEL (in red):
       tp        ga        gw
val:   93.4      1.36      8
sd:    17.8      0.244     14.7

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   93.4      1.36      8         3.79      7.36
sd:    NA        NA        NA        NA        NA

       CNST         HILL         GNLS
AIC:   242          229.01       233.01
PROB:  0            0.88         0.12
RMSE:  69.44        50.72        50.72

MAX_MEAN: 93.1       MAX_MED: 100        BMAD: 24.1

COFF: 72.3    HIT-CALL: 1     FITC: 41    ACTP: 1
```



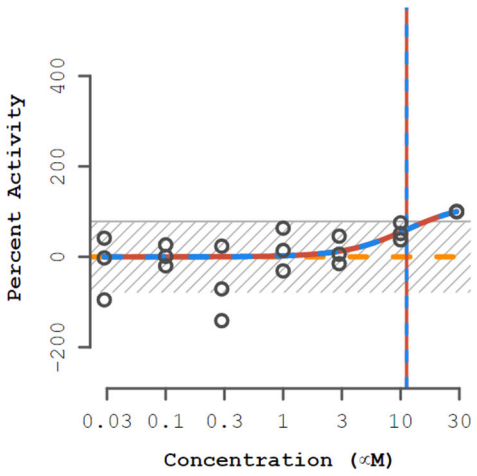

```
ASSAY:    AEID2508 (CCTE_Shafer_MEA_dev_interburst_inter

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:     42623671

HILL MODEL (in red):
       tp        ga        gw
val:   95.2      1.14      8
sd:    8.2       0.512     29.3

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   95.2      1.14      8         3.16      13.4
sd:    NA        NA        NA        NA        NA

       CNST         HILL         GNLS
AIC:   232.64       203.34       207.34
PROB:  0            0.88         0.12
RMSE:  56.55        24.81        24.81

MAX_MEAN: 95.4       MAX_MED: 100        BMAD: 21.5

COFF: 64.6    HIT-CALL: 1     FITC: 41    ACTP: 1
```



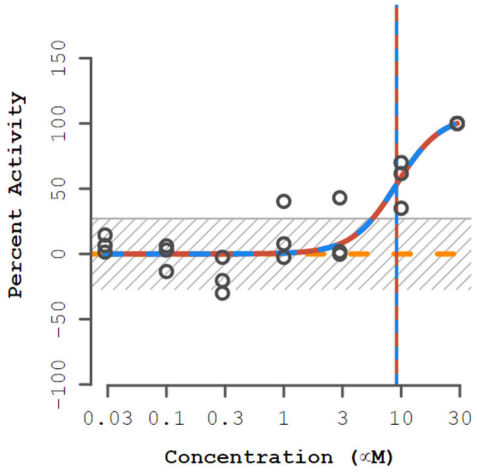

```
ASSAY:   AEID2508 (CCTE_Shafer_MEA_dev_interburst_inter

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:    42623882

HILL MODEL (in red):
      tp       ga       gw
val:  120      1.06     1.4
sd:   48.4     0.357    0.764

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  120      1.06     1.4      2.71     8.07
sd:   48.4     0.357    0.764    12400    81200

      CNST       HILL       GNLS
AIC:  230.08     217.65     221.65
PROB: 0          0.88       0.12
RMSE: 52.33      34.23      34.23

MAX_MEAN: 100      MAX_MED: 100      BMAD: 21.5

COFF: 64.6   HIT-CALL: 1    FITC: 42   ACTP: 1
```



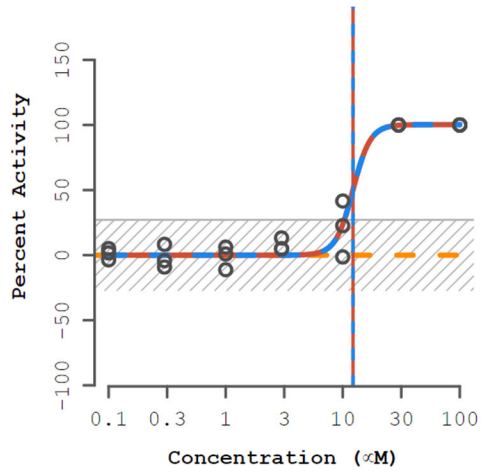

```
ASSAY:   AEID2510 (CCTE_Shafer_MEA_dev_network_spike_nu

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    42624577

HILL MODEL (in red):
      tp       ga       gw
val:  100      1.08     8
sd:   8.6      0.745    72

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  100      1.08     7.95     2.26     12.3
sd:   NaN      NaN      NaN      NaN      NaN

      CNST       HILL       GNLS
AIC:  237.9      211.56     215.56
PROB: 0          0.88       0.12
RMSE: 62.98      32.47      32.47

MAX_MEAN: 100      MAX_MED: 100      BMAD: 26.1

COFF: 78.3   HIT-CALL: 1    FITC: 41   ACTP: 1
```



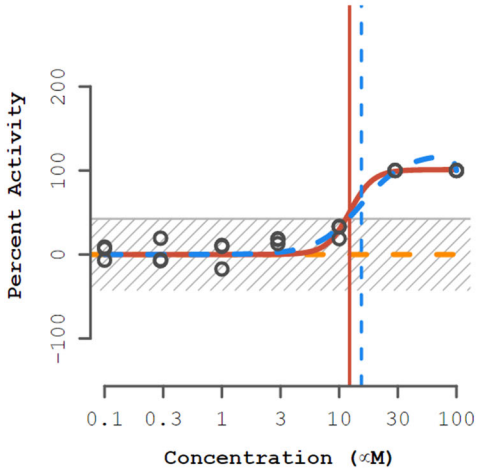

```
ASSAY:   AEID2510 (CCTE_Shafer_MEA_dev_network_spike_nu

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:    42624635

HILL MODEL (in red):
      tp       ga       gw
val:  120      1.05     1.64
sd:   71.7     0.434    1.81

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  120      1.05     1.64     2.79     10.8
sd:   NA       NA       NA       NA       NA

      CNST       HILL       GNLS
AIC:  237.52     224.62     228.62
PROB: 0          0.88       0.12
RMSE: 63.51      46.39      46.39

MAX_MEAN: 100      MAX_MED: 100      BMAD: 26.1

COFF: 78.3   HIT-CALL: 1    FITC: 42   ACTP: 1
```



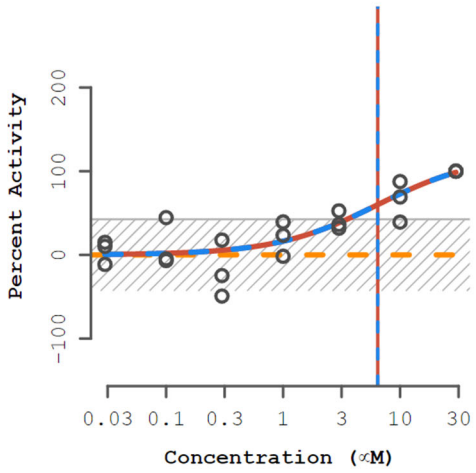

```
ASSAY:   AEID2512 (CCTE_Shafer_MEA_dev_network_spike_pe

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:    42625592

HILL MODEL (in red):
      tp       ga       gw
val:  107      0.961    2.29
sd:   14.4     0.0878   1.31

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  107      0.961    2.29     2.56     15.9
sd:   NA       NA       NA       NA       NA

      CNST       HILL       GNLS
AIC:  221.45     182.86     186.86
PROB: 0          0.88       0.12
RMSE: 46.43      16.16      16.16

MAX_MEAN: 100      MAX_MED: 100      BMAD: 9.04

COFF: 27.1   HIT-CALL: 1    FITC: 42   ACTP: 1
```



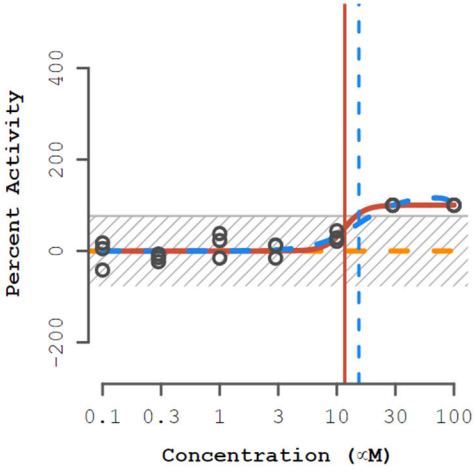

```
ASSAY:    AEID2512 (CCTE_Shafer_MEA_dev_network_spike_pe

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:     42625811

HILL MODEL (in red):
       tp        ga        gw
val:   100       1.09      5.85
sd:    2.69      0.0881    5.8

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   100       1.09      5.85      2.79      12
sd:    NaN       NaN       NaN       NaN       NaN

       CNST         HILL         GNLS
AIC:   228.37       154.67       158.67
PROB:  0            0.88         0.12
RMSE:  54.7         8.58         8.58

MAX_MEAN: 100       MAX_MED: 100       BMAD: 9.04

COFF: 27.1    HIT-CALL: 1    FITC: 41    ACTP: 1
```



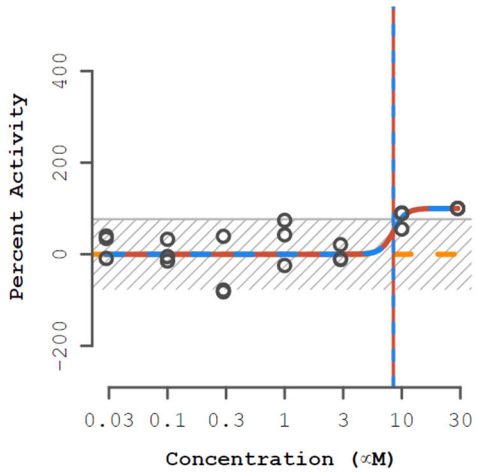

```
ASSAY:    AEID2514 (CCTE_Shafer_MEA_dev_spike_duration_m

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:     42626373

HILL MODEL (in red):
       tp        ga        gw
val:   101       1.09      4.34
sd:    4.55      0.0657    3.06

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   120       1.19      2.31      2.04      18
sd:    46.9      0.204     1.74      0.0099    22

       CNST         HILL         GNLS
AIC:   229.73       166.22       168.31
PROB:  0            0.74         0.26
RMSE:  55.45        10.06        9.61

MAX_MEAN: 100       MAX_MED: 100       BMAD: 14.2

COFF: 42.5    HIT-CALL: 1    FITC: 41    ACTP: 1
```



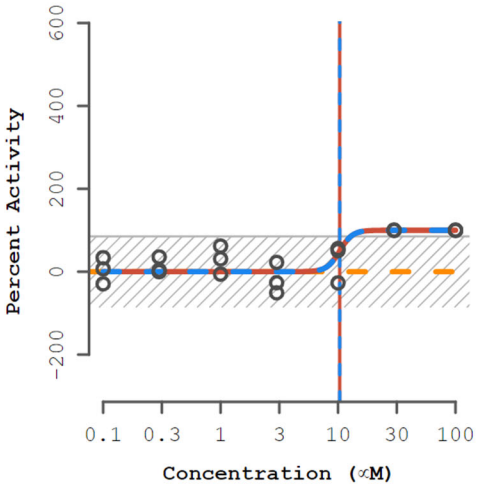

```
ASSAY:   AEID2514 (CCTE_Shafer_MEA_dev_spike_duration_m

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:    42626685

HILL MODEL (in red):
      tp       ga       gw
val:  120      0.809    0.992
sd:   33.8     0.346    0.413

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  120      0.809    0.992    2.17     10.3
sd:   33.9     0.346    0.413    287      4290

      CNST       HILL       GNLS
AIC:  228.92     193.59     197.59
PROB: 0          0.88       0.12
RMSE: 52.12      20.65      20.65

MAX_MEAN: 100      MAX_MED: 100      BMAD: 14.2

COFF: 42.5   HIT-CALL: 1    FITC: 42   ACTP: 1
```



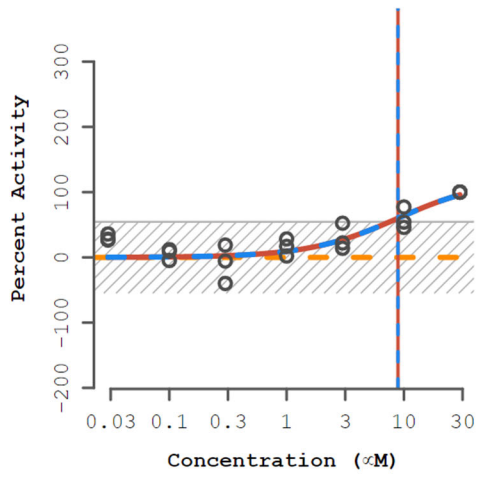

```
ASSAY:   AEID2516 (CCTE_Shafer_MEA_dev_network_spike_du

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    42627245

HILL MODEL (in red):
      tp       ga       gw
val:  100      1.07     5.22
sd:   6.82     0.167    12.4

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  120      1.19     2.39     2.08     9.06
sd:   79.4     0.374    2.79     0.0924   25.4

      CNST       HILL       GNLS
AIC:  232.3      185.56     189.28
PROB: 0          0.87       0.13
RMSE: 57.26      16.79      16.73

MAX_MEAN: 100      MAX_MED: 100      BMAD: 25.5

COFF: 76.6   HIT-CALL: 1    FITC: 41   ACTP: 1
```



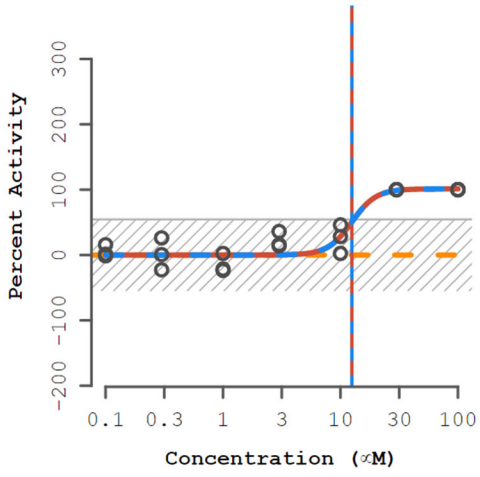

```
ASSAY:   AEID2516 (CCTE_Shafer_MEA_dev_network_spike_du:

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:    42627323

HILL MODEL (in red):
      tp       ga       gw
val:  100      0.928    8
sd:   14.5     0.764    85.2

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  100      0.928    8        2.9      17.4
sd:   NA       NA       NA       NA       NA

      CNST       HILL       GNLS
AIC:  236.28     218.33     222.33
PROB: 0          0.88       0.12
RMSE: 60.22      36.47      36.47

MAX_MEAN: 100      MAX_MED: 100      BMAD: 25.5

COFF: 76.6   HIT-CALL: 1    FITC: 41   ACTP: 1
```



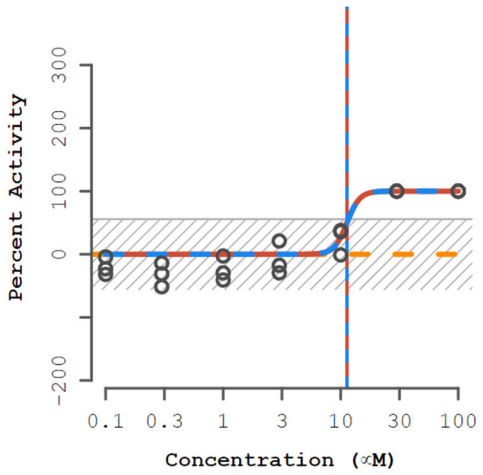

```
ASSAY:   AEID2518 (CCTE_Shafer_MEA_dev_inter_network_sp:

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    42628508

HILL MODEL (in red):
      tp       ga       gw
val:  100      1.01     8
sd:   7.64     0.0873   45.1

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  100      1.01     8        2.42     8.67
sd:   12       0.0879   45       64.7     1250

      CNST       HILL       GNLS
AIC:  236.48     207.16     211.16
PROB: 0          0.88       0.12
RMSE: 61.09      28.55      28.55

MAX_MEAN: 100      MAX_MED: 100      BMAD: 28.4

COFF: 85.2   HIT-CALL: 1    FITC: 37   ACTP: 1
```



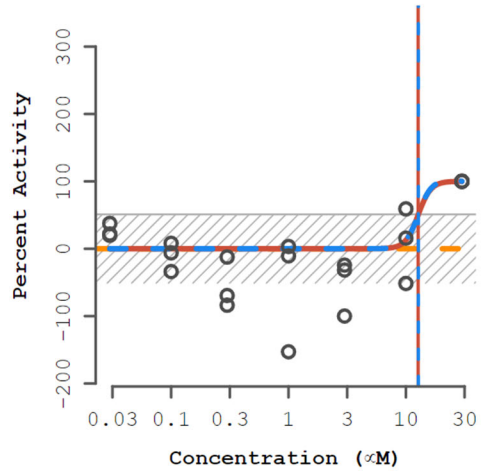

```
ASSAY:    AEID2520 (CCTE_Shafer_MEA_dev_per_network_spik

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:     42629339

HILL MODEL (in red):
       tp        ga        gw
val:   120       0.949     1.13
sd:    27.9      0.241     0.397

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   120       0.949     1.13      2.84      18
sd:    NA        NA        NA        NA        NA

       CNST         HILL         GNLS
AIC:   225.54       190.81       194.81
PROB:  0            0.88         0.12
RMSE:  49.04        18.31        18.31

MAX_MEAN: 100       MAX_MED: 100       BMAD: 18.2

COFF: 54.5    HIT-CALL: 1     FITC: 42    ACTP: 1
```



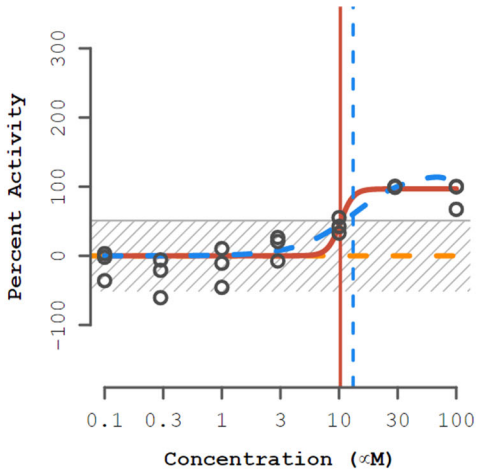

```
ASSAY:    AEID2520 (CCTE_Shafer_MEA_dev_per_network_spik

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:     42629571

HILL MODEL (in red):
       tp        ga        gw
val:   101       1.1       4.22
sd:    6.76      0.0909    3.93

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   101       1.1       4.22      3.97      7.03
sd:    NaN       NaN       NaN       NaN       NaN

       CNST         HILL         GNLS
AIC:   231.32       184.03       188.03
PROB:  0            0.88         0.12
RMSE:  56.55        15.73        15.73

MAX_MEAN: 100       MAX_MED: 100       BMAD: 18.2

COFF: 54.5    HIT-CALL: 1     FITC: 41    ACTP: 1
```



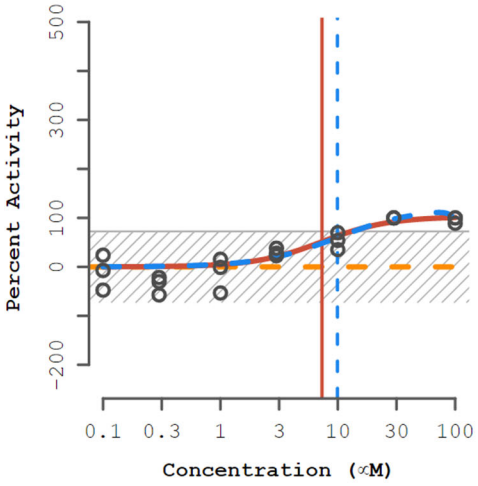

```
ASSAY:   AEID2522 (CCTE_Shafer_MEA_dev_per_network_spik

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    42630417

HILL MODEL (in red):
      tp       ga       gw
val:  100      1.05     8
sd:   6.93     0.511    76.1

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  100      1.05     8        4        1.67
sd:   NaN      NaN      NaN      NaN      NaN

      CNST       HILL       GNLS
AIC:  234        199.19     203.19
PROB: 0          0.88       0.12
RMSE: 58.64      22.37      22.37

MAX_MEAN: 100      MAX_MED: 100      BMAD: 18.6

COFF: 55.7   HIT-CALL: 1   FITC: 41   ACTP: 1
```



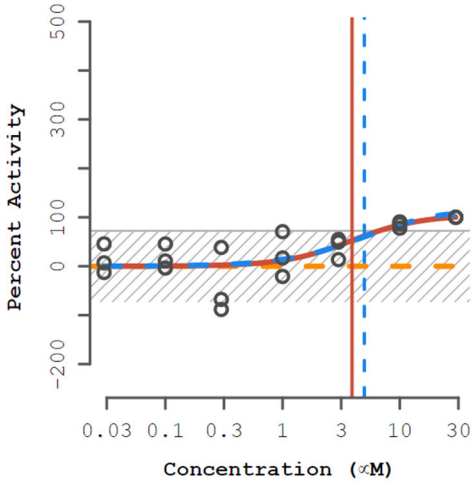

```
ASSAY:   AEID2524 (CCTE_Shafer_MEA_dev_correlation_coef

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:    42631127

HILL MODEL (in red):
      tp       ga       gw
val:  100      1.1      8
sd:   21.5     1.28     96.6

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  102      1.11     7.93     1.59     15.6
sd:   NaN      NaN      NaN      NaN      NaN

      CNST       HILL       GNLS
AIC:  237.35     230.65     234.65
PROB: 0.03       0.85       0.12
RMSE: 64.37      52.06      52.06

MAX_MEAN: 100      MAX_MED: 100      BMAD: 17

COFF: 51     HIT-CALL: 1   FITC: 41   ACTP: 0.97
```



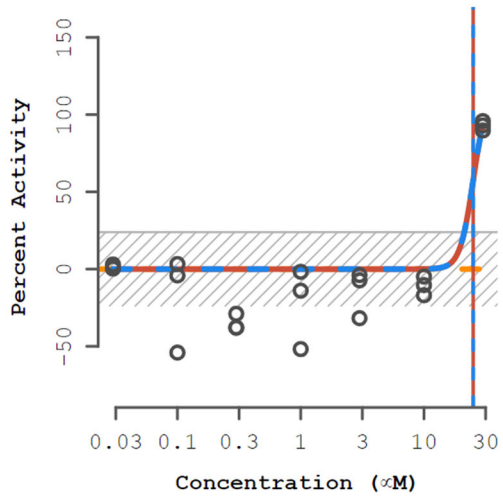

```
ASSAY:   AEID2524 (CCTE_Shafer_MEA_dev_correlation_coef:

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    42631350

HILL MODEL (in red):
      tp       ga       gw
val:  96.7     1.01     8
sd:   6.32     0.116    80.9

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  120      1.12     1.84     2.06     8.76
sd:   34.2     0.186    0.933    0.104    15.3

      CNST       HILL       GNLS
AIC:  233.33     195.01     197.49
PROB: 0          0.78       0.22
RMSE: 57.41      21.94      21.43

MAX_MEAN: 99.7      MAX_MED: 100       BMAD: 17

COFF: 51     HIT-CALL: 1    FITC: 41   ACTP: 1
```



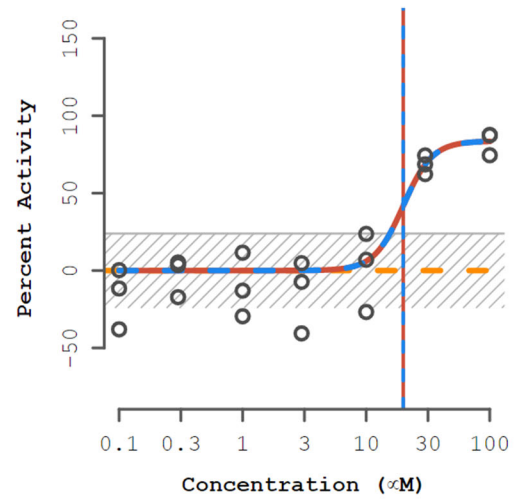

```
ASSAY:   AEID2526 (CCTE_Shafer_MEA_dev_mutual_informati

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    42631705

HILL MODEL (in red):
      tp       ga       gw
val:  103      0.865    1.53
sd:   9.87     0.135    0.422

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  120      0.997    1.33     2.08     9.61
sd:   NaN      NaN      NaN      NaN      NaN

      CNST       HILL       GNLS
AIC:  237.42     200.72     203.74
PROB: 0          0.82       0.18
RMSE: 61.81      24.7       24.52

MAX_MEAN: 99.9      MAX_MED: 100       BMAD: 24.1

COFF: 72.4   HIT-CALL: 1    FITC: 41   ACTP: 1
```



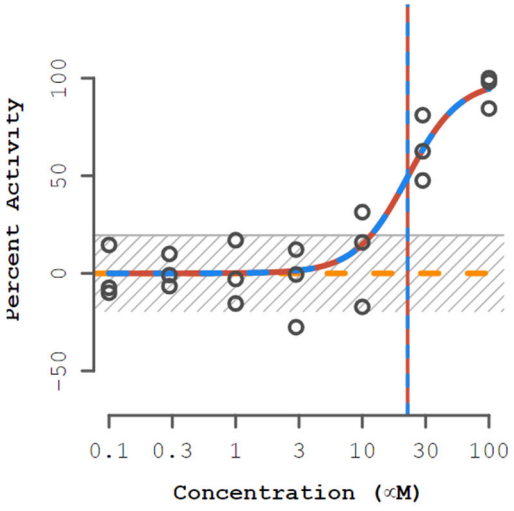

```
ASSAY:   AEID2526 (CCTE_Shafer_MEA_dev_mutual_informati

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:    42631718

HILL MODEL (in red):
      tp       ga       gw
val:  105      0.593    1.49
sd:   21.3     0.21     0.982

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  120      0.698    1.26     1.54     17.9
sd:   NaN      NaN      NaN      NaN      NaN

      CNST       HILL       GNLS
AIC:  238.05     213.97     217.96
PROB: 0          0.88       0.12
RMSE: 62.25      34.16      34.25

MAX_MEAN: 100      MAX_MED: 100      BMAD: 24.1

COFF: 72.4   HIT-CALL: 1    FITC: 41   ACTP: 1
```



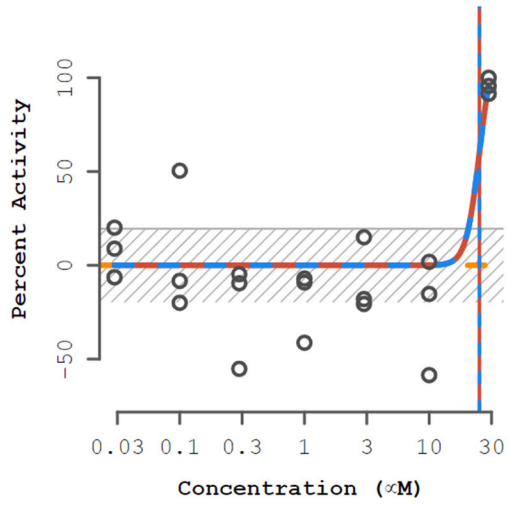

```
ASSAY:   AEID2529 (CCTE_Shafer_MEA_dev_LDH_dn)

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:    42632533

HILL MODEL (in red):
      tp       ga       gw
val:  115      1.4      8
sd:   265      0.578    12.2

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  115      1.4      8        2.1      10.8
sd:   258      0.563    12.1     1240     21400

      CNST       HILL       GNLS
AIC:  216.75     197.38     201.38
PROB: 0          0.88       0.12
RMSE: 41.94      22.97      22.97

MAX_MEAN: 92.8      MAX_MED: 93      BMAD: 7.96

COFF: 23.9   HIT-CALL: 1    FITC: 42   ACTP: 1
```




```
ASSAY:    AEID2529 (CCTE_Shafer_MEA_dev_LDH_dn)

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:     42632781

HILL MODEL (in red):
       tp        ga        gw
val:   83.6      1.3       3.69
sd:    7.41      0.099     1.8

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   83.6      1.3       3.69      3.8       16.1
sd:    NA        NA        NA        NA        NA

       CNST      HILL      GNLS
AIC:   221.46    186.53    190.53
PROB:  0         0.88      0.12
RMSE:  44.25     17.45     17.45

MAX_MEAN: 83.2      MAX_MED: 87.4      BMAD: 7.96

COFF: 23.9    HIT-CALL: 1    FITC: 41    ACTP: 1
```




```
ASSAY:    AEID2530 (CCTE_Shafer_MEA_dev_AB_dn)

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:     42633266

HILL MODEL (in red):
       tp        ga        gw
val:   98.8      1.36      2.11
sd:    10.3      0.0927    0.813

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   98.8      1.36      2.11      2.51      13.3
sd:    10.3      0.0927    0.813     578       15000

       CNST      HILL      GNLS
AIC:   220.36    178.99    182.99
PROB:  0         0.88      0.12
RMSE:  45.25     13.82     13.82

MAX_MEAN: 94.2      MAX_MED: 98.1      BMAD: 6.5

COFF: 19.5    HIT-CALL: 1    FITC: 41    ACTP: 1
```



ASSAY: AEID2530 (CCTE_Shafer_MEA_dev_AB_dn)

NAME: Malathion
CHID: 20791 CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID: 42633314

HILL MODEL (in red):

	tp	ga	gw
val:	120	1.4	8
sd:	315	0.628	13.7

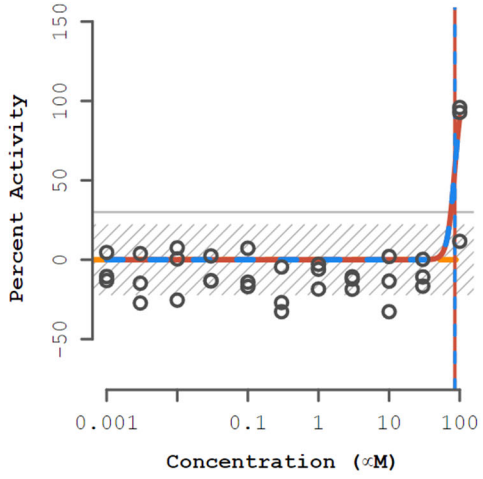
GAIN-LOSS MODEL (in blue):

	tp	ga	gw	la	lw
val:	120	1.4	8	3.42	8.87
sd:	NA	NA	NA	NA	NA

	CNST	HILL	GNLS
AIC:	219.23	201.41	205.41
PROB:	0	0.88	0.12
RMSE:	44.01	25.09	25.09

MAX_MEAN: 95.7 MAX_MED: 95.6 BMAD: 6.5

COFF: 19.5 HIT-CALL: 1 FITC: 42 ACTP: 1



ASSAY: AEID2777 (CCTE_Mundy_HCI_Cortical_NOG_BPCount_

NAME: Malathion
CHID: 20791 CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID: 43468773

HILL MODEL (in red):

	tp	ga	gw
val:	115	1.93	8
sd:	139	0.171	19.1

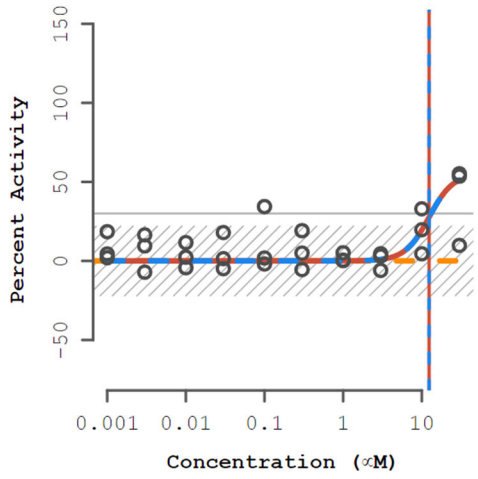
GAIN-LOSS MODEL (in blue):

	tp	ga	gw	la	lw
val:	115	1.93	8	3.89	4.65
sd:	139	0.172	19.1	51100	125000

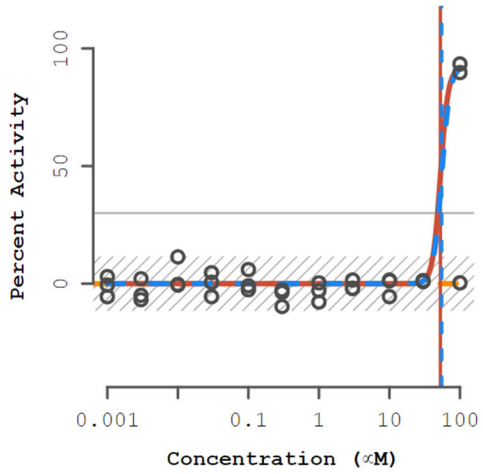
	CNST	HILL	GNLS
AIC:	301.75	293.72	297.72
PROB:	0.02	0.87	0.12
RMSE:	27.68	20.22	20.22

MAX_MEAN: 66.8 MAX_MED: 92.8 BMAD: 7.34

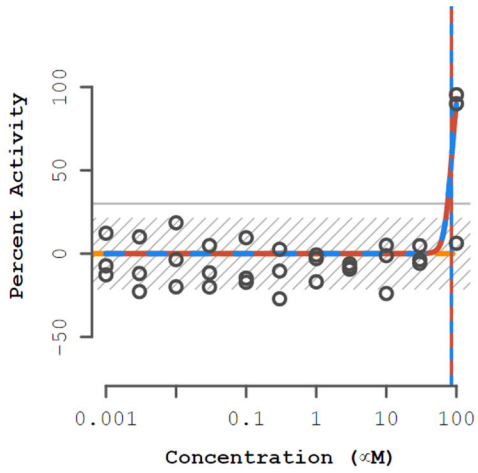
COFF: 30 HIT-CALL: 1 FITC: 42 ACTP: 0.98



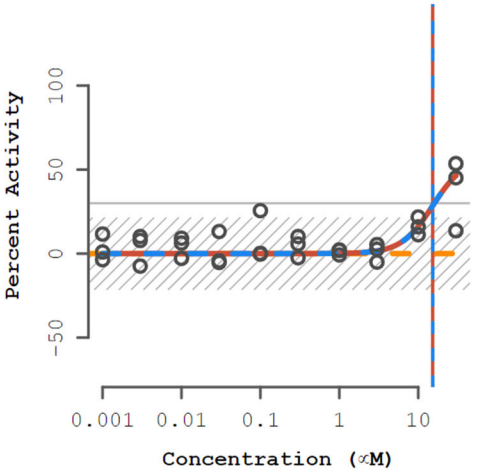

```
ASSAY:   AEID2777 (CCTE_Mundy_HCI_Cortical_NOG_BPCount_

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:    43468797

HILL MODEL (in red):
      tp       ga       gw
val:  55.3     1.09     2.84
sd:   27.9     0.309    5.14

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  55.3     1.09     2.84     2.67     8.41
sd:   NaN      NaN      NaN      NaN      NaN

      CNST       HILL       GNLS
AIC:  255.52     241.6      245.6
PROB: 0          0.88       0.12
RMSE: 18.7       13.07      13.07

MAX_MEAN: 39.5      MAX_MED: 53.6      BMAD: 7.34

COFF: 30     HIT-CALL: 1    FITC: 42   ACTP: 1
```



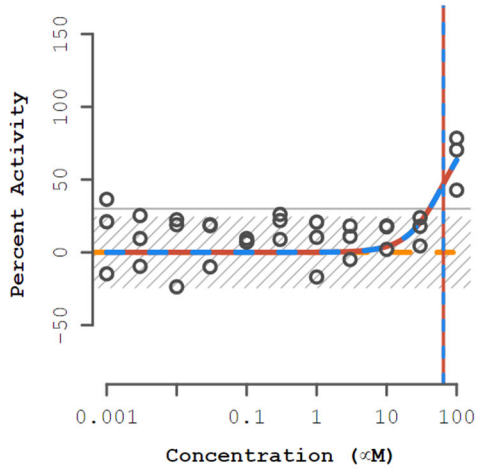

```
ASSAY:   AEID2778 (CCTE_Mundy_HCI_Cortical_NOG_NeuriteC

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    43468693

HILL MODEL (in red):
      tp       ga       gw
val:  91.8     1.72     7.89
sd:   19.1     0.836    26.5

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  92.5     1.74     7.35     2.33     9.46
sd:   NaN      NaN      NaN      NaN      NaN

      CNST       HILL       GNLS
AIC:  239.51     221.21     225.21
PROB: 0          0.88       0.12
RMSE: 22.94      16.37      16.37

MAX_MEAN: 61.2      MAX_MED: 89.8      BMAD: 3.8

COFF: 30     HIT-CALL: 1    FITC: 41   ACTP: 1
```



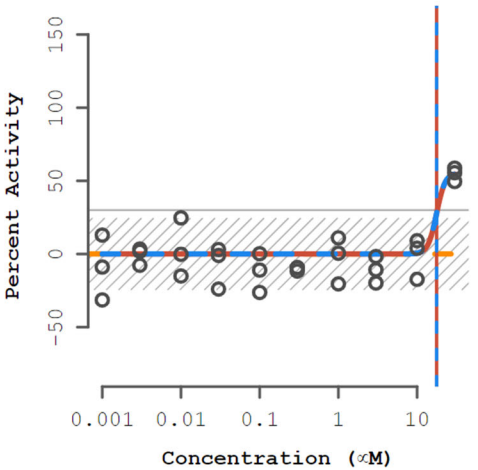

```
ASSAY:   AEID2779 (CCTE_Mundy_HCI_Cortical_NOG_NeuriteL

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    43468415

HILL MODEL (in red):
      tp       ga       gw
val:  114      1.93     8
sd:   NaN      NaN      NaN

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  114      1.93     7.91     3.99     1.71
sd:   NaN      NaN      NaN      NaN      NaN

      CNST       HILL       GNLS
AIC:  292.49     284.15     288.15
PROB: 0.01       0.87       0.12
RMSE: 25.99      19.06      19.06

MAX_MEAN: 63.9      MAX_MED: 90        BMAD: 7.09

COFF: 30     HIT-CALL: 1    FITC: 42   ACTP: 0.99
```



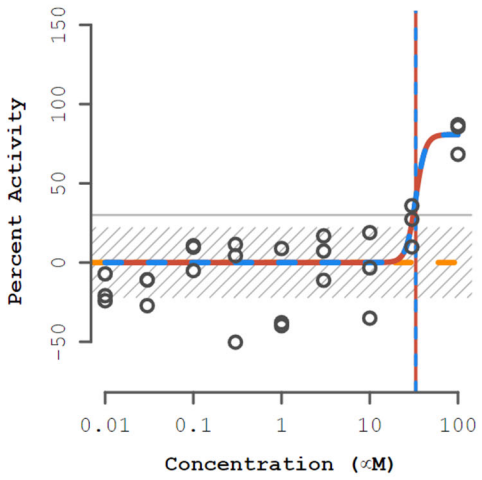

```
ASSAY:   AEID2779 (CCTE_Mundy_HCI_Cortical_NOG_NeuriteL

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:    43468500

HILL MODEL (in red):
      tp       ga       gw
val:  56.9     1.18     2.18
sd:   43.1     0.447    2.62

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  57.7     1.18     2.19     3.47     0.95
sd:   NaN      NaN      NaN      NaN      NaN

      CNST       HILL       GNLS
AIC:  243.49     222.04     226.04
PROB: 0          0.88       0.12
RMSE: 15.85      9.6        9.6

MAX_MEAN: 37.4      MAX_MED: 45.1      BMAD: 7.09

COFF: 30     HIT-CALL: 1    FITC: 42   ACTP: 1
```



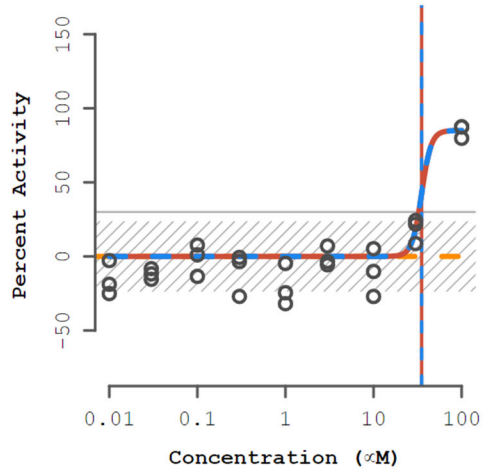

```
ASSAY:   AEID2780 (CCTE_Mundy_HCI_Cortical_NOG_NeuronCo

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    43468272

HILL MODEL (in red):
      tp       ga       gw
val:  94.1     1.81     1.66
sd:   64.5     0.417    1.47

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  94.1     1.81     1.66     3.89     6.91
sd:   64.5     0.417    1.47     142000   328000

      CNST       HILL       GNLS
AIC:  308.44     292        296
PROB: 0          0.88       0.12
RMSE: 25.97      16.61      16.61

MAX_MEAN: 63.9      MAX_MED: 70.4      BMAD: 8.12

COFF: 30     HIT-CALL: 1    FITC: 42   ACTP: 1
```



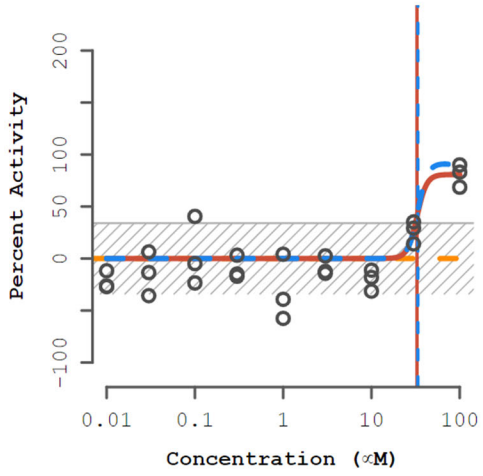

```
ASSAY:   AEID2780 (CCTE_Mundy_HCI_Cortical_NOG_NeuronCo

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): EPAPLT0167G08
M4ID:    43468355

HILL MODEL (in red):
      tp       ga       gw
val:  55.5     1.25     7.96
sd:   172      6.06     188

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  55.5     1.25     7.97     3.28     8.8
sd:   NA       NA       NA       NA       NA

      CNST       HILL       GNLS
AIC:  268.84     249.79     253.79
PROB: 0          0.88       0.12
RMSE: 21.82      13.35      13.35

MAX_MEAN: 54.6      MAX_MED: 55.6      BMAD: 8.12

COFF: 30     HIT-CALL: 1    FITC: 41   ACTP: 1
```



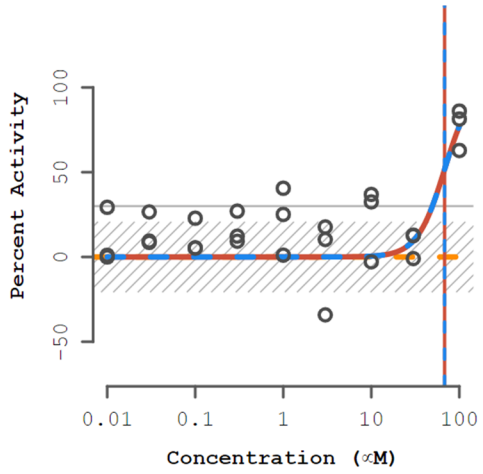

```
ASSAY:    AEID2781 (CCTE_Mundy_HCI_Cortical_Synap&Neur_M

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:     43470179

HILL MODEL (in red):
       tp        ga        gw
val:   80.8      1.52      8
sd:    9.06      0.251     45.3

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   80.8      1.52      8         2.32      12.6
sd:    6.88      0.251     45.3      3.83      681

       CNST         HILL         GNLS
AIC:   267.86       246.37       250.37
PROB:  0            0.88         0.12
RMSE:  34.34        19.91        19.91

MAX_MEAN: 80.3       MAX_MED: 85.7       BMAD: 7.35

COFF: 30      HIT-CALL: 1     FITC: 41    ACTP: 1
```



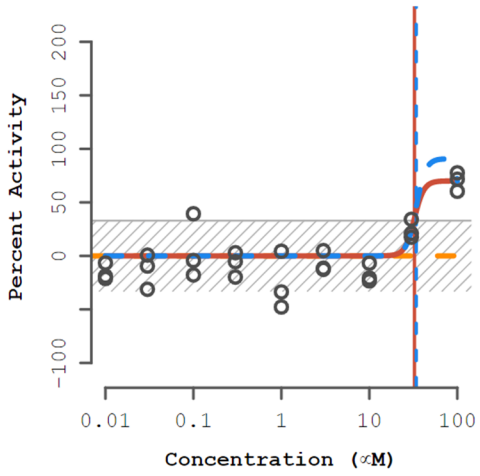

```
ASSAY:    AEID2784 (CCTE_Mundy_HCI_Cortical_Synap&Neur_M

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:     43469856

HILL MODEL (in red):
       tp        ga        gw
val:   85        1.55      8
sd:    6.19      0.615     71.1

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   85        1.55      8         3.9       9.24
sd:    NA        NA        NA        NA        NA

       CNST         HILL         GNLS
AIC:   258.71       226.7        230.7
PROB:  0            0.88         0.12
RMSE:  32.09        13.87        13.87

MAX_MEAN: 84.9       MAX_MED: 87.4       BMAD: 7.81

COFF: 30      HIT-CALL: 1     FITC: 41    ACTP: 1
```



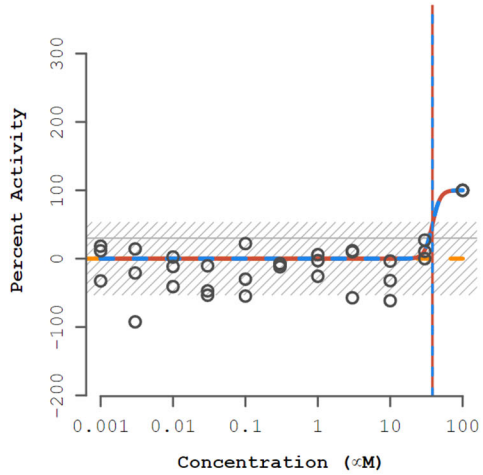

```
ASSAY:    AEID2786 (CCTE_Mundy_HCI_Cortical_Synap&Neur_M

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:     43469569

HILL MODEL (in red):
       tp        ga        gw
val:   80.8      1.52      8
sd:    9.94      0.126     25

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   91.5      1.53      8         2.09      9.74
sd:    111       0.176     24.5      0.0426    23.1

       CNST         HILL         GNLS
AIC:   271.19       251.74       255.74
PROB:  0            0.88         0.12
RMSE:  35.74        21.9         21.9

MAX_MEAN: 80.6       MAX_MED: 83.1       BMAD: 11.3

COFF: 34      HIT-CALL: 1     FITC: 41    ACTP: 1
```



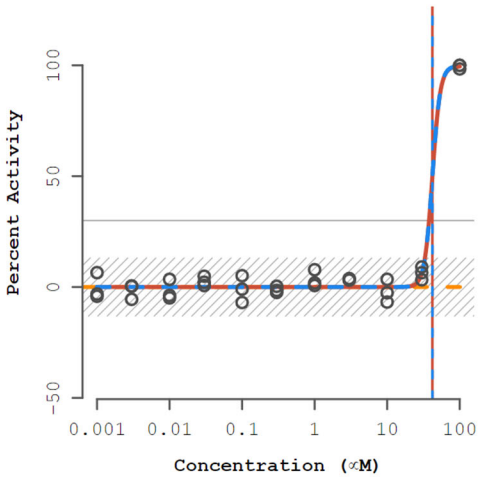

```
ASSAY:    AEID2787 (CCTE_Mundy_HCI_Cortical_Synap&Neur_M

NAME:     Malathion
CHID:     20791    CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:     43469172

HILL MODEL (in red):
       tp        ga        gw
val:   103       1.83      2.8
sd:    174       0.766     4.62

GAIN-LOSS MODEL (in blue):
       tp        ga        gw        la        lw
val:   103       1.83      2.8       3.93      17.7
sd:    NA        NA        NA        NA        NA

       CNST         HILL         GNLS
AIC:   263.49       245.67       249.67
PROB:  0            0.88         0.12
RMSE:  32.11        19.17        19.17

MAX_MEAN: 76.7       MAX_MED: 81.4       BMAD: 6.84

COFF: 30      HIT-CALL: 1     FITC: 42    ACTP: 1
```



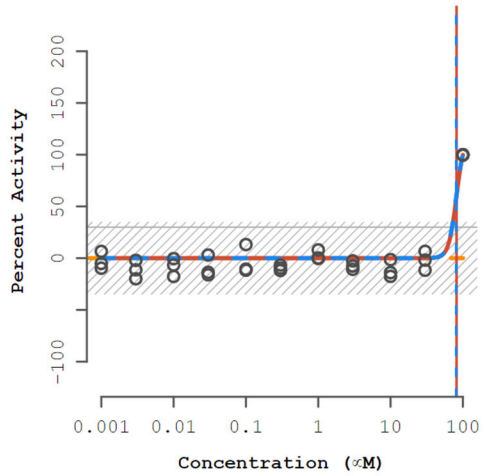

```
ASSAY:   AEID2788 (CCTE_Mundy_HCI_Cortical_Synap&Neur_M

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    43470336

HILL MODEL (in red):
      tp       ga       gw
val:  70       1.51     8
sd:   8.17     0.11     23.8

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  90.9     1.53     8        2.03     16.6
sd:   NaN      NaN      NaN      NaN      NaN

      CNST       HILL       GNLS
AIC:  263.08     242.21     246.21
PROB: 0          0.88       0.12
RMSE: 30.85      18.56      18.56

MAX_MEAN: 69.9      MAX_MED: 71.7      BMAD: 11

COFF: 32.9   HIT-CALL: 1    FITC: 41   ACTP: 1
```



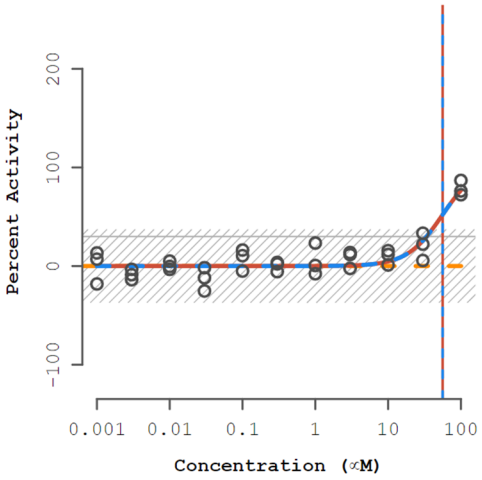

```
ASSAY:   AEID1 (CCTE_Mundy_HCI_CDI_iCell_Neurons_BPCount_loss

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    15

HILL MODEL (in red):
      tp       ga       gw
val:  100      1.58     8
sd:   13.4     1.54     115

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  100      1.58     8        2.98     8.37
sd:   NaN      NaN      NaN      NaN      NaN

      CNST       HILL       GNLS
AIC:  342.92     326.03     330.03
PROB: 0          0.88       0.12
RMSE: 43.43      31.02      31.02

MAX_MEAN: 100       MAX_MED: 100       BMAD: 17.7

COFF: 30     HIT-CALL: 1    FITC: 41   ACTP: 1

FLAGS: 6; 10
```




```
ASSAY:   AEID2 (CCTE_Mundy_HCI_CDI_iCell_Neurons_NeuriteCount

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    43

HILL MODEL (in red):
      tp       ga       gw
val:  99.6     1.62     8
sd:   NaN      NaN      NaN

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  99.8     1.62     7.98     2.26     10
sd:   NaN      NaN      NaN      NaN      NaN

      CNST       HILL       GNLS
AIC:  265.72     190.9      194.9
PROB: 0          0.88       0.12
RMSE: 30.27      3.68       3.68

MAX_MEAN: 99.5      MAX_MED: 100       BMAD: 4.37

COFF: 30     HIT-CALL: 1    FITC: 41   ACTP: 1

FLAGS: 6
```

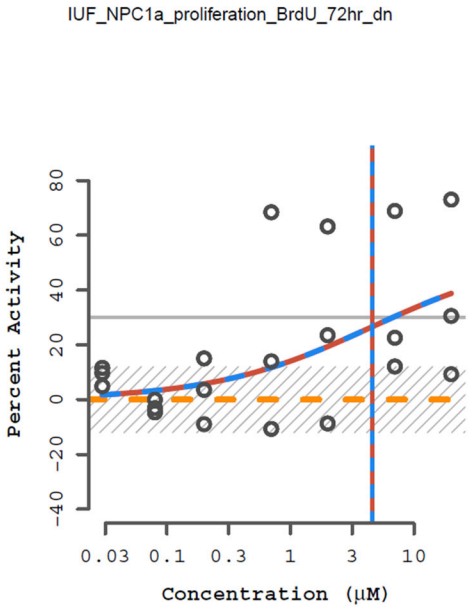



```
ASSAY:   AEID3 (CCTE_Mundy_HCI_CDI_iCell_Neurons_NeuriteLengt

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    71

HILL MODEL (in red):
      tp       ga       gw
val:  120      1.91     7.95
sd:   365      0.228    70.5

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  118      1.91     8        4        14
sd:   NA       NA       NA       NA       NA

      CNST       HILL       GNLS
AIC:  297.68     255.65     259.65
PROB: 0          0.88       0.12
RMSE: 31.64      9.75       9.75

MAX_MEAN: 99.8      MAX_MED: 100       BMAD: 11.6

COFF: 30     HIT-CALL: 1    FITC: 42   ACTP: 1

FLAGS: 6
```

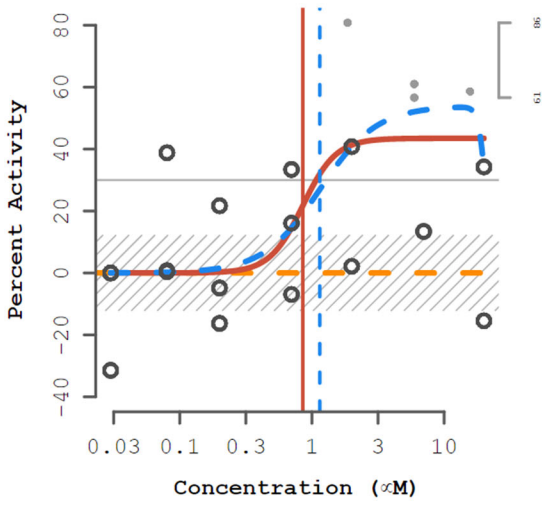



```
ASSAY:   AEID4 (CCTE_Mundy_HCI_CDI_iCell_Neurons_NeuronCount_

NAME:    Malathion
CHID:    20791   CASRN: 121-75-5
SPID(S): TT0000177D02
M4ID:    99

HILL MODEL (in red):
      tp       ga       gw
val:  104      1.75     1.78
sd:   37.8     0.226    0.865

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  104      1.75     1.78     3.99     5.97
sd:   37.8     0.226    0.864    194000   586000

      CNST       HILL       GNLS
AIC:  299.01     260.02     264.02
PROB: 0          0.88       0.12
RMSE: 26.76      10.72      10.72

MAX_MEAN: 78.4      MAX_MED: 76.2      BMAD: 12.3

COFF: 30     HIT-CALL: 1    FITC: 42   ACTP: 1

FLAGS: 6
```

Appendix C. Concentration Response Curve for Malaoxon in Human Cell Lines.

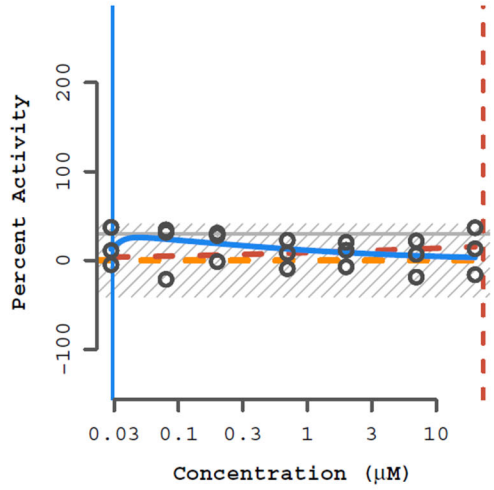



```
ASSAY:   AEID2771 (IUF_NPC1a_proliferation_BrdU_72hr_dn)

NAME:    Malaoxon
CHID:    20790   CASRN: 1634-78-2
SPID(S): EX000566
M4ID:    43530656

HILL MODEL (in red):
      tp       ga       gw
val:  53.2     0.661    0.671
sd:   54.9     1.58     0.539

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  53.2     0.661    0.671    2.76     4.83
sd:   55       1.58     0.539    918      3050

      CNST       HILL       GNLS
AIC:  930.71     878.14     882.14
PROB: 0          0.88       0.12
RMSE: 33.69      23.61      23.61

MAX_MEAN: 42.9      MAX_MED: 30.6      BMAD: 4.02

COFF: 30     HIT-CALL: 1    FITC: 42   ACTP: 1

FLAGS: 11; 17
```



IUF_NPC4_neurite_length_120hr_dn

```
ASSAY:   AEID2774 (IUF_NPC1_viability_72hr_up)

NAME:    Malaoxon
CHID:    20790   CASRN: 1634-78-2
SPID(S): EX000566
M4ID:    43530999  BRK

HILL MODEL (in red):
      tp       ga       gw
val:  43.5     -0.0689  3.45
sd:   5.29     0.13     3.97

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  53.9     0.0595   2.02     1.32     15.8
sd:   8.76     0.154    1.13     0.0501   56.7

      CNST       HILL       GNLS
AIC:  1054.4     989.09     989.59
PROB: 0          0.56       0.44
RMSE: 36.2       25.18      24.61

MAX_MEAN: 46.9      MAX_MED: 61.3      BMAD: 4.04

COFF: 30     HIT-CALL: 1    FITC: 41   ACTP: 1
```




```
ASSAY:   AEID2948 (IUF_NPC4_neurite_length_120hr_dn)

NAME:    Malaoxon
CHID:    20790   CASRN: 1634-78-2
SPID(S): EX000566
M4ID:    43532517

HILL MODEL (in red):
      tp       ga       gw
val:  31.4     1.36     0.3
sd:   NaN      NaN      NaN

GAIN-LOSS MODEL (in blue):
      tp       ga       gw       la       lw
val:  44.7     -1.51    8        -0.968   0.478
sd:   33.9     0.0839   43.9     1.32     0.259

      CNST       HILL       GNLS
AIC:  960.35     946.42     930.35
PROB: 0          0          1
RMSE: 21.64      19.7       18.52

MAX_MEAN: 19.1      MAX_MED: 31.6      BMAD: 13.7

COFF: 30     HIT-CALL: 1    FITC: 50   ACTP: 1

FLAGS: 11; 17
```

Appendix D. Summary of Available Epidemiology Studies Investigating Associations Between Malathion and Potential Neurodevelopmental Outcomes

Outcome	Outcome Test(s)	Exposure Measurement	Study Design	Result	Study Population	Study Quality	Author
Developmental	Brazelton Neonatal Behavioral Assessment Scale (BNBAS)	Malathion dicarboxylic acid (MDA) detected in urine collected 3rd trimester of pregnancy	Cohort	Evidence of a moderately strong to strong association between prenatal urinary MDA levels above the limit of detection (given only 21% detection vs. 79% non-detection) and the number of abnormal reflexes, part of the central nervous system function, in neonatal babies using the Poisson regression (counts of abnormal reflexes; RR: 2.24; 95% CI: 1.55, 3.24 n =242) and multivariable logistic regression model (dichotomized the counts of abnormal reflexes; OR: 3.6; 95% CI: 1.5, 8.8) No evidence of a statistically significant change was observed for habituation, orientation, motor performance; regulation of state, range of state, levels of stimulation, and autonomic stability in newborns following prenatal exposure to malathion in the multivariable linear model (habituation β: 0.440, 95% CI: -0.145, 1.025 with n = 148; orientation β: -0.100, 95% CI: -0.597, 0.405 with n = 240; motor β: -0.050, 95% CI: -0.233, 0.156 with n = 257; range of state β: -0.040, 95% CI: -0.281, 0.199 with n = 256; regulation of state β: -0.090, 95% CI: -0.480, 0.303 with n = 256; autonomic stability β: 0.090, 95% CI: -0.274, 0.463 with n = 256).	Children's Environmental Health Study Mount Sinai hospital in New York City	Moderate	Engel et al., 2007
Social-Emotional	Child Behavior Checklist (CBCL, Spanish version)	Malathion dicarboxylic acid (MDA) detected in urine	Cross-sectional	No evidence of a significant association was observed between urinary MDA metabolite levels and any of the behavior score outcomes (both syndrome scores and composite scores) among male adolescents in either tertile (T2 or T3) relative to referent tertile (T1) ($-1.42 \leq \beta \leq 2.19$; all 95% CIs encompassed the null value of 0, p-trends > 0.05).	Environment and Childhood (INMA) Granada, Spain	Low	Rodriguez-Carrillo et al., 2022
Developmental Social-Emotional	Bayley Scales of Infant Development [Mental Development (MDI) and Psychomotor Development (PDI) Indices] combined with the Child Behavior Checklist (CBCL)	Malathion dicarboxylic acid (MDA) detected in urine	Cohort	No evidence of a significant association was observed between prenatal urinary MDA metabolite levels in either exposure category (< median detected, ≥ median detected) and children's MDI scores at their 6-month, 12-month and 24-months visits, relative to the referent ($-1.09 \leq \beta s \leq 2.40$; all CIs encompassed the null value of 0). Similarly, for children's PDI scores at their 6-month, 12-month, and 24-month visits, no evidence of a significant association was observed for	CHAMACOS Salinas Valley, CA, US Mother-child pairs CHAMACOS	Moderate	Eskenazi et al., 2007

Outcome	Outcome Test(s)	Exposure Measurement	Study Design	Result	Study Population	Study Quality	Author
				prenatal urinary MDA metabolite levels in either exposure category (< median detected, ≥ median detected), relative to the referent category (-1.45 ≤ βs ≤ 0.75; all CIs encompassed the null value of 0).			
Learning and Memory, General Intelligence/IQ, Executive Functioning, Processing Speed	Wechsler Intelligence Scale for Children at 7 years	GIS-based - agricultural pesticide application within 1-km of maternal residence at birth and agricultural	Cohort	Without adjustment for exposure to other pesticides, no evidence of a significant association was reported between a standard deviation increase in malathion application within 1-km of the maternal residence during pregnancy and any of the IQ scales tested at 7-years of age - Verbal Comprehension, Full-Scale IQ, Working Memory, Processing Speed, and Perceptual Reasoning (-1.30 < β < 0.80; all CIs encompassed the null value of 0; all p-values > 0.05). With adjustment for exposure to other pesticides, no evidence of a significant association was reported between a standard deviation increase in malathion application at 1-km from the maternal residence during pregnancy and Full-Scale IQ at 7-years of age from multiple pesticide models (β = 0.20; 95% CI: -1.70, 2.1). Authors did not report results for any of the other outcomes (Working Memory, Processing Speed, Verbal Comprehension, and Perceptual Reasoning).			Gunier et al., 2017
Social-Emotional	Neurobehavioral development and emotional problems Behavior Assessment System for Children, 2nd edition at 16 and 18 years (n=593)	GIS-based – Assessment agricultural pesticide application within 1-km of maternal residence during and after pregnancy.	Cohort	Evidence of a borderline statistically significant association between a 2-fold increase in malathion applications within 1 km of the residence *during childhood (0-5 yrs)* and decreased *maternal-reported* attention problems among all children at age 16 and 18 years (β = -0.9; 95% CrI:-1.8, 0.0). Evidence of a borderline statistically significant association between a 2-fold increase in malathion applications within 1 km of the residence *during childhood (0-5 yrs)* and decreased *youth-reported* attention problems for girls at age 16 and 18 years (β = -1.1; 95% CI: -2.1, 0.0). Youth-reported externalizing problems were not tested. No evidence of significant associations observed between two-fold increase in malathion applications *during pregnancy* and any investigated outcome (either maternal- or youth-reported).		Moderate	Hyland et al., 2021

Outcome	Outcome Test(s)	Exposure Measurement	Study Design	Result	Study Population	Study Quality	Author
	Neurobehavioral development and emotional problems with Adverse Childhood Experiences at 16 and 18 years	GIS-based Assessment agricultural pesticide application within 1-km of maternal residence after pregnancy.	Cohort	For *maternal reported* neurobehavioral development and emotional problems among children at 16 and 18 years of age, evidence of a statistically significant association between a 2-fold increase in malathion use within a 1 km radius of residence during pregnancy and internalizing problems was reported only among participants with high ACEs (β: 1.9, 95% CrI: 0.2, 3.7 n = 127 in the analysis using BASC-2 scores assessed at 16- and 18-year visits; β: 2.6, 95% CrI: 0.6, 4.6 n = 127 in the analysis using only BASC-2 scores assessed at 18-year visits), and among boys (not girls) with high ACEs (β: 2.8, 95% CrI: 0.1, 5.6; n = 47 in the analysis using BASC-2 scores assessed at ages 16- and 18- year visits). For *youth reported* neurobehavioral development and emotional problems among children at 16 and 18 years of age, evidence of a statistically significant association was reported between a 2-fold increase in malathion use within a 1 km radius of residence during pregnancy and internalizing problems only among participants with high ACEs (β: 2.1, 95% CrI: 0.4, 3.8; n = 127 in the analysis using BASC-2 scores assessed at 16- and 18-year visits; β: 2.4, 95% CrI: 0.5, 4.3; n = 127 in the analysis using only BASC-2 scores assessed at 18-year visits), and among boys (not girls) with high ACEs (β: 4.9, 95% CrI: 1.9, 8.0; n = 47 boys in the analysis using BASC-2 scores assessed at ages 16- and 18- year visits). Since a statistically significant association was observed only in boys with high ACEs but not in boys with low ACEs, a borderline statistically significant association was also reported among all boys together (β: 1.2, 95% CrI: 0.0, 2.5; n = 216 in the analysis using BASC-2 scores assessed at ages 16- and 18- year visits). Evidence of borderline statistically significant associations between a 2-fold increase in malathion use within a 1 km radius of residence during pregnancy and hyperactivity at high ACE level (β: 1.7 (95% CrI 0.1, 3.2; n = 127), in the analysis using all data) and attention problems at high ACE level (β: 3.0 (95% CrI 0.1, 6.0; n = 47) in the analysis using only boy data);			Hyland et al., 2022

Outcome	Outcome Test(s)	Exposure Measurement	Study Design	Result	Study Population	Study Quality	Author
				however, these significant association occurrences were considered as random chances due to a large number of comparisons and different analyses for each outcome endpoint.			
Risk Taking Behavior	Risk-Taking Behavior and Adverse Childhood Experiences from childhood at 18 years	GIS-based Assessment agricultural pesticide application within 1-km of maternal residence after pregnancy.	Cohort	Among children with high ACEs, evidence of a slight positive association was reported between a 2-fold increase in malathion use within 1 km of the residence during pregnancy for number of unique delinquent acts and for frequency of delinquent acts at age 18 years (IRR = 1.16; 95% CrI: 1.03, 1.31; 95% CrI does not include the null value of 1.0; IRR = 1.30; 95% CrI: 1.11, 1.51; 95% CrI does not include the null value of 1.0). No evidence of a significant positive association was observed for children with low ACEs or among all children for number of unique delinquent acts and for frequency of delinquent acts at age 18 years (0.91 ≤ IRR ≤ 1.05; all CrIs include the null value of 1.0). No evidence of a significant positive association was observed for any other outcome (police encounters, any delinquent act, or risk count) among all children and among children with high or low ACEs at 18 years of age, following a two-fold increase in wind-adjusted malathion use within 1-km of residence during pregnancy (0.92 ≤ IRR ≤ 1.24; all CrIs include the null value of 1.0).			Gunier et al., 2022
Autism Spectrum Disorder (2 studies)	Social Responsiveness Scale, Version 2 (SRS-2) at age 14 (parent), Behavior Assessment System for Children, Version 2 (BASC-2) at age 7, 10½, and 14, (parent, teacher at age 7) Evaluación Neuropsicológica Infantil (ENI) Facial Expression Recognition Test at age 9, and the NEPSY-II Affect Recognition subtest at age 12	The amount of malathion applied within each 2.59 square-km weighted by the proportion of agricultural land area within the 1 km buffer was used to determine exposure for each residence. Average pesticide use during the entire pregnancy was determined by summing the trimester-specific estimates of malathion applied and dividing by the number of trimesters included in the assessment for each residence.	Cohort	Results were presented as change in outcome score for a 10-fold increase in prenatal OP pesticide use, along with corresponding 95% CIs and p-values. No evidence of a significant association was reported between a 10-fold increase in prenatal malathion exposure use within 1-km of residence during pregnancy and the 14 years of age SRS-2 Total T-score (β = 0.50; 95% CI: –0.70, 1.80; with n = 235), the SRS-2 DSM-V compatible Social Communication and Interaction (SCI) T-score (β = 0.40; 95% CI: -0.90, 1.60; with n = 235), and the SRS-2 DSM-V compatible Restricted and Repetitive Behaviors (RRB) T-score (β = 0.90; 95% CI: –0.30, 2.10; with n = 235). For reference, the T-score standardized mean=50 (SD= 10) and higher SRS score indicates more ASD-related traits. Similar results were reported in a sensitivity analysis using 10-fold increase of malathion		Low	Sagiv et al., 2018

Outcome	Outcome Test(s)	Exposure Measurement	Study Design	Result	Study Population	Study Quality	Author
				use within 3-km of residence during pregnancy. No evidence of a significant association was reported between a 10-fold increase in prenatal malathion exposure use within 1 km of residence during pregnancy and BASC-2 Social Skills T-score Teacher report at 7-y (β = -1.70; 95% CI: –5.40, 2.00; with n = 270), BASC-2 Social Skills T-sore- Parent Report at 7-y, 10 ½, and 14 y (β = -0.70; 95% CI: –1.90, 0.50; with n = 354), and Affect Recognition ENI at 9-y (β = -0.10; 95% CI: –0.20, 0.10; with n = 310), and Affect Recognition NEPSY-II at 12-y (β = 0.10; 95% CI: -0.50, 0.60; with n = 307). For reference, lower scores are consistent with more ASD-related traits and BASC-2 T-score standardized mean=50 (SD= 10); ENI mean=6.6 (SD=1.2); NEPSY-II mean=26.6 (SD=3.6).			
	Autism Spectrum Disorder with intellectual disability (Diagnostic and Statistical Manual of Mental Disorders, fourth edition, revised)	GIS-based - Agricultural pesticide application within 2- km of maternal residence before, during and after pregnancy	Case-control	No evidence of a significant positive association between malathion exposure during any timepoint (3 months pre-pregnancy, pregnancy, first year of life) and autism spectrum disorder with or without intellectual disability when adjusted for other pesticides (0.73 < all other ORs < 1.29; all 95% CIs encompassed the null value of 1.0).	Mother-child pairs in San Joaquin Valley, CA, US	Low	von Ehrenstein et al., 2019
Neural Tube Defects	California Birth Defects Monitoring Program Case Definition for Neural Tube Defects	GIS-based Assessment agricultural pesticide application within 1,000-m of maternal residence during pregnancy	Case-control	No evidence of a positive association between exposure to malathion applied within 1000 m of maternal residence and neural tube defects (Single-Pesticide Model – OR=1.0; 95% CI 0.5, 2.3; Multiple-Pesticide Model – OR=1.0; 95% CI 0.4, 2.7; Hierarchical Logistic Regression (multiple pesticide) Model – OR=0.9; 5% CI 0.5-1.9; n=13 malathion exposed cases).	CA, US Birth Defects Registry	Moderate	Rull et al., 2006
Cerebral Palsy	California Department of Development Services (DDS) diagnostic records	GIS-based Assessment agricultural pesticide application within 2000-m of maternal residence at birth	Population-based case-control	No evidence of a significant positive association for ambient malathion exposure during the first trimester of pregnancy and cerebral palsy among females for either model adjustment (OR = 1.10; 95% CI: 0.97, 1.24; additionally adjusted for pesticides – OR = 1.18; 95% CI: 0.97, 1.24; with n = 443 exposed female cases) or among males (OR = 0.95: 95% CI: 0.86, 1.06; additionally adjusted for pesticides – OR = 1.04; 95% CI: 0.92, 1.17; with n = 529 exposed male cases).	Mothers and babies born in San Joaquin Valley of California	Moderate	Liew et al., 2020