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New worm on the block: Planarians in (neuro)toxicology

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Abstract

Traditional mammalian testing is too time- and cost-intensive to keep up with the large number of environmental chemicals. This has led to a dearth of information about the potential adverse effects these chemicals have, especially on the developing brain. Thus, there is an urgent need for rapid and cost-effective neurotoxicity and developmental neurotoxicity testing. Because of the complexity of the brain, metabolically competent organismal models are necessary to understand the effects of chemicals on nervous system development and function on a systems level. In this overview, we showcase asexual freshwater planarians as an alternative invertebrate ("non-animal") organismal model for neurotoxicology research. Planarians have long been used to study the effects of chemicals on regeneration and behavior. But they have only recently moved back into the spotlight because modern molecular and computational approaches now enable quantitative high-content and high-throughput toxicity studies. Here, we present a short history of the usage of planarians in toxicology research, highlight current techniques to qualitatively and quantitatively measure toxicity in planarians, and discuss how to further promote this non-animal organismal system into mainstream toxicology research. The articles in this collection will help work towards this goal by providing detailed protocols that can be adopted by the community to standardize planarian toxicity testing.

Keywords

invertebrate; developmental neurotoxicity; behavior; new approach methodology; screening

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CONFLICT OF INTEREST STATEMENT:

EMC is the founder of Inveritek, LLC, which offers planarian screening commercially. EMC and DI have a pending patent application for planarian HTS.

INTRODUCTION

Chemical safety has traditionally been established using animal tests. While considered the gold standard for decades, mammalian testing has major limitations. It is incredibly slow and expensive – taking months or even years to complete and costing hundreds of thousands to a million USD per chemical, depending on the test (Meigs et al., 2018). Thus, only a small number (estimated at ~20% (Meigs et al., 2018)) of chemicals currently in the environment have been thoroughly evaluated for safety. In addition to the extreme time and cost burden, animal tests have been shown to have limited predictive power for human health. Comparisons with data from human clinical trials have shown that animal toxicity tests are only predictive of human toxicity up to 50% of the time; thus, no better than random chance (Van Norman, 2019). Lack of toxicity in animal tests (even nonhuman primates) is not predictive of lack of toxicity in humans, suggesting that animal tests are not protective of human health (Bailey et al., 2014, 2015). Animal studies also suffer from a lack of reproducibility. By analyzing a database of more than 800,000 standard animal toxicity studies across 350 chemicals, Meigs et al. (Meigs et al., 2018) found that results on a specific chemical (i.e., identified as toxic or not) in the same animal species were only reproducible 70% of the time. Interspecies differences among animal models further complicate matters (Bailey et al., 2015; Pham et al., 2020). Together with ethical concerns, these economic and scientific reasons have convinced the various stakeholders - government, industry, academia, non-profit, and the public - to replace animal tests in toxicology and pharmacology with quicker, cheaper and more predictive non-animal test methods. Integrated strategies are being developed to effectively use the information gained from these new approach methodologies to be predictive of human health (Middleton et al., 2022; Rovida et al., 2015). For certain toxicities, a combination of in vitro and in silico test methods may be able to completely replace animal tests and have already been accepted for regulatory purposes, e.g., skin sensitization (Rovida et al., 2015; OECD, 2021). However, for many types of toxicities, a total replacement of animal tests is not yet possible.

One of the most challenging areas has been neurotoxicity (NT) and developmental neurotoxicity (DNT) testing (Sachana et al., 2021). Because of the complexity of the brain and the plasticity of developmental processes, one can only fully understand the effect of chemicals on brain function and development when studied in an organismal context. Driven by these challenges, a major effort is underway to develop invertebrate organismal models that could replace or at least reduce testing in mammals and other vertebrates (e.g., fish and frog). The nematode *Caenorhabditis elegans* and embryos and larvae of the zebrafish *Danio rerio* have emerged as popular alternatives to mammals for developmental toxicity testing. Although zebrafish are vertebrates, the larval stage prior to 5 days post fertilization is considered a non-animal model for regulatory purposes. Both nematodes and developing zebrafish have major strengths for developmental and reproductive toxicity testing (e.g., reviewed in (Boyd et al., 2012; Tejeda-Benitez and Olivero-Verbel, 2016) and (Shen and Zuo, 2020; He et al., 2014), respectively).

Here, we showcase freshwater planarians as a competitive alternative model for NT/DNT testing, with a special focus on the asexual species *Dugesia japonica*, which has been shown to be the best suited for rapid behavioral screening applications (Ireland et al.,

2020; Blackiston et al., 2010). We provide a brief background on the use of planarian neuroregeneration to study neurodevelopment, followed by a historical overview of how planarians have been used for toxicological applications. Next, we discuss how recent technological advancements have opened the door for quantitative behavioral studies which can be backed up by various molecular/cellular techniques to provide mechanistic insight. Lastly, we discuss what is still needed to further promote this non-animal organismal system into mainstream toxicology research.

PLANARIAN NEUROREGENERATION AS A MODEL FOR NEURODEVELOPMENT

Planarians are few mm long flatworms with a simple anatomy (Figure 1). They have an anterior-posterior (head-tail) and a ventral-dorsal body axis. Much of the planarian's body is taken up by a multi-branched gut. Like nematodes, planarians lack a circulatory system; their large surface area and extensive digestive system allow for adequate oxygen and nutrient distribution. Because planarians are aquatic organisms, they can be easily exposed to chemical solutions, which are absorbed through the skin and/or the pharynx (eating tube) (Kapu and Schaeffer, 1991; Balestrini et al., 2014). When amputated, planarians regenerate the missing body parts within 1–2 weeks. Due to their size being significantly larger compared to that of nematodes, planarian regeneration can be easily observed without the need for specialized equipment. These features have made planarians popular for chemical exposure studies to investigate (neuro-)development (Best and Morita, 1982).

The planarian nervous system is of intermediate complexity when compared to that of other non-animal organismal models (about 2,000–10,000 neurons (Brown et al., 2018)). The planarian central nervous system consists of a cephalic ganglion (brain) in the head and two ventral nerve cords that are connected by multiple commissures. These structures can be further subdivided into specific functional and molecular regions, demonstrating its molecular complexity (Fraguas et al., 2012; Cebrià et al., 2002). The planarian nervous system shares key characteristics with the vertebrate nervous system, including all key neurotransmitters (reviewed in (Ross et al., 2017)). All planarian neuronal genes identified to date have human homologs (Mineta et al., 2003). After amputation or asexual division, the planarian tail fragment has to regenerate a new brain, which occurs *de novo* and independently from the intact nerve cords (Fraguas et al., 2012) through similar processes as in vertebrate neurodevelopment (e.g., stem cell proliferation, migration and differentiation, synaptogenesis, and network formation) (reviewed in detail in (Ross et al., 2017)). Thus, planarian neurobiology and neuroregeneration are sufficiently conserved to provide insight into the possible effects of xenobiotics on human brain development.

Planarians exhibit a wide range of behaviors that can be used as a readout of brain function. Deviations from typical behavior in response to chemical exposure can be used as an indicator of effects on the nervous system. Planarians have three characteristic gaits. The default form of locomotion is smooth, ciliary-driven motion termed 'gliding' (Cochet-Escartin et al., 2015). When cilia are impaired, planarians switch to a musculature-driven gait called 'peristalsis' that is characterized by an anterior-posterior traveling wave.

Peristalsis is significantly slower than gliding (Cochet-Escartin et al., 2015). Finally, when exposed to certain noxious stimuli (e.g., amputation, noxious heat, or certain chemicals like allyl isothiocyanate), planarians 'scrunch' (Cochet-Escartin et al., 2015; Sabry et al., 2019). Scrunching is a cilia-independent form of musculature-driven locomotion that is characterized by asymmetric body shape oscillation with a species-specific frequency (Cochet-Escartin et al., 2015). Planarians have been shown to respond robustly to physical stimuli such as light, temperature, and textures (Cochet-Escartin et al., 2015; Pearl, 1903; Inoue et al., 2004, 2014, 2015). Thus, in addition to changes in motility or gait, behavioral readouts in planarians can include measures of the directionality or rate of movement, e.g., moving away from light (Inoue et al., 2004) or the rate of reaction to noxious heat (Ireland et al., 2020). Combinatorial assays using simultaneous exposure to multiple stimuli (e.g., light and temperature) can be used to assess higher brain functions, such as decision making (Inoue et al., 2015). Finally, because flatworms are soft-bodied worms, they can exhibit dramatic body shape changes, e.g., contraction, hyperextension, c-shaped or corkscrew, in response to chemical exposure (reviewed in (Hagstrom et al., 2016)). This contrasts nematodes, which have a cuticle that serves as an exoskeleton and gives roundworms their characteristic worm shape. Taken together, the multiple behavioral and body shape readouts observed in planarians offer a uniquely rich spectrum of quantifiable phenotypic readouts for assaying neurotoxic effects.

BRIEF HISTORY OF FRESHWATER PLANARIANS IN TOXICOLOGY RESEARCH

Freshwater planarians have fascinated researchers for centuries for their amazing ability to regenerate from tiny fragments into new, functional organisms within 1–2 weeks (Figure 2, reviewed in (Ivankovic et al., 2019)). Planarian research first blossomed at the turn of the 20th century. Early research focused on characterizing the physiology and behaviors of these flatworms, with and without various perturbations (Child, 1930; Fries, 1928; Pearl, 1903; Child, 1911). During this time, the promise of planarians to be used for toxicology studies, especially for the study of developmental toxicants became apparent. Their amenability to chemical perturbation and observation without a microscope, coupled with their high propensity to regenerate, made planarians a popular early model for investigations of chemical exposure on regeneration and neuronal control of behavior. Often, planarians were cut into various pieces, these pieces were exposed to the chemical of choice, and qualitative observations of planarian health and regeneration progress were made, as for example in (Child, 1911). Typical observations of toxic effects included abnormal head morphology, lesions, improper regeneration of the eyes or auricles, pharynx extrusion and qualitative descriptions of abnormal behaviors (Best and Morita, 1982).

While many early studies were conducted with species native to North America (e.g., *Dugesia dorotocephala*), the growing availability of different planarian species have allowed for the use of non-American species such as *Schmidtea mediterranea* (native to Europe) and *Dugesia japonica* (native to Asia). These latter two species have increasingly grown in popularity for toxicology research – reflected in the increased number of publications (from none in 2009 to 12 in 2019) - through the availability of sequenced genomes (Grohme et al.,

2018; Tian et al., 2022; Robb et al., 2008) and transcriptomes (Rozanski et al., 2019) and the development of molecular and cellular biology tools, such as RNA interference (RNAi) (Shibata and Agata, 2018; Newmark et al., 2003), that allow for mechanistic studies.

Over the years, several valuable resources have been introduced with the goal of standardizing toxicological measures across planarian studies. For example, a qualitative scoring system for common planarian toxicological endpoints was first introduced by Grebe and Schaeffer (Grebe and Schaeffer, 1991), and later modified by Wu et al. (Wu et al., 2012). This scoring system has been used by several subsequent studies but remains to be standardized across the field. Similarly, the introduction of the planarian locomotor velocity (pLMV (Raffa et al., 2001)) assay provided an accessible method to quantify planarian motility and is still widely used. With the toxicology field moving away from mammalian and vertebrate testing, teaching the next generation of scientists about the value of alternative invertebrate models, such as planarians, for toxicology and pharmacology studies have become increasingly important. Easy-to implement laboratory protocols have been developed which allow for low-cost, hands-on experiments for school and college students (Pagán et al., 2009; Stowell et al., 2021).

Recent advances in molecular and cellular biology techniques, behavioral imaging, and quantitative computational methods have reignited the excitement about planarians for studying chemical toxicity. The application of these tools has allowed planarian toxicology studies to evolve from primarily qualitative observations to quantitative analyses of specific phenotypes, which can be anchored to their molecular mechanisms (as for example in (Ireland et al., 2022b; Balestrini et al., 2014; Hagstrom et al., 2018; Dong et al., 2021)). The wide array of stereotypical behaviors exhibited by these worms has successfully been exploited in neurotoxicology studies to uncover the effects of chemicals on brain function (reviewed in (Hagstrom et al., 2016; Wu and Li, 2018)). Neurobiological (e.g., (Inoue et al., 2014; Sabry et al., 2019)) and pharmacological (e.g., (Venturini et al., 1989)) studies have begun to connect some of these behaviors to their molecular mediators, opening the door for mechanistic phenotypic profiling (Ireland et al., 2022b).

AN OLD WORM MEETS MODERN TOOLS: AUTOMATED QUANTITATIVE BEHAVIORAL SCREENING TO ASSAY NT/DNT

Planarians can display a plethora of body shape changes and behaviors that can be triggered through physical or chemical stimuli (reviewed in (Hagstrom et al., 2016; Grebe and Schaeffer, 1991). While postures and behaviors have historically been scored manually, such as using pLMV (Raffa et al., 2001)), recent studies have employed computer vision and automated object tracking to quantify a subset of planarian behaviors (Talbot and Schötz, 2011; Zhang et al., 2019a; Ireland et al., 2022b; Blackiston et al., 2010). The planarians' pigmented body is easily detectable on a bright background and standard thresholding algorithms can be used to isolate the worm. Center of mass (COM) tracking can then be used to determine the worm's spatial position as a function of time and allows for the calculation of motility descriptors, such as speed, time spent resting, locomotor bursts or spatial exploration (Ireland et al., 2022b). COM tracking also enables visualization of

the planarian trajectory, e.g., color-coded by time, to visualize directional movements, as observed in light, thermal or electrical gradients (Hagstrom et al., 2015; Sabry et al., 2022; Inoue et al., 2004, 2014) (Figure 3). The response to these gradients is frequently quantified as the percent of time spent in a specific portion of the test arena (Inoue et al., 2004, 2014). The trajectories can also be used to determine the number of turns or head wiggles, as in (Talbot and Schötz, 2011). Protocols for how to track the COM of planarians using freeware and requiring no coding experience have recently been made available (Sabry et al., 2020; Stowell et al., 2021; Inoue and Agata, 2022).

Quantitative shape descriptors can provide additional insight into planarian behavior. For example, by fitting the planarian body with an ellipse and plotting the major axis (approximating the planarian's body length in straight motion) as a function of time, it can be determined whether a planarian exhibits ciliary gliding or a form of muscle-driven locomotion (scrunching or peristalsis). Gliding shows minimal changes in body length whereas scrunching is characterized by periodic length oscillations (Sabry et al., 2020; Cochet-Escartin et al., 2015). Scrunching has also been distinguished from gliding using shape mode analysis (Werner et al., 2014).

While many planarian studies have relied on bulk or low-throughput studies, recent studies have begun to utilize behavioral screening in multi-well plates to allow for simultaneous tracking of multiple planarians to increase throughput (Hagstrom et al., 2015; Zhang et al., 2019a, 2019b; Ireland et al., 2020, 2022b). Multi-well screening can be used to evaluate effects on morphology, general locomotion, and behavioral responses to certain physical stimuli (e.g., light, temperature) (Zhang et al., 2019b, 2019a; Ireland et al., 2022b, 2020). Because the small size of the individual wells makes some spatial gradients difficult to establish, temporal changes in stimuli can be used instead. For example, to test the planarian's response to noxious heat, the temperature is gradually increased until the threshold for scrunching induction is reached. This allows researchers to study the rate and strength of the worm's response to heat in addition to assaying their ability to scrunch (Ireland et al., 2020).

Taken together, these stimulated behaviors can provide a more nuanced understanding of chemical effects on neuronal function than can be obtained from locomotion alone. For example, alterations to the stereotypical noxious heat response are a sensitive readout of neurotoxicity that can often occur in the absence of general locomotor defects (Zhang et al., 2019a, 2019b; Hagstrom et al., 2018; Ireland et al., 2022b; Bayingana et al., 2022). Furthermore, some phenotypic readouts have begun to be linked to molecular pathways; for example, we have found that cholinergic agents frequently disrupt stickiness and noxious heat sensation (Hagstrom et al., 2018; Ireland et al., 2022b). Pharmacological studies have revealed that activation of D1 dopamine receptors leads to screw-like hyperkinesia, whereas activation of D2 dopamine receptors leads to c-shapes (Venturini et al., 1989). RNAi-mediated knockdown has been used to identify important mediators of specific behaviors, such as TRPM in regulating thermotaxis (Inoue et al., 2014) and GABAergic neurons in regulating phototaxis (Nishimura et al., 2008). An in depth review of RNAi-mediated behavioral phenotypes can be found in (Ross et al., 2017). Despite this progress, most of the molecular mechanisms underlying these shape changes or behaviors remain to

be investigated. There is a need for high-content mechanistic studies to link phenotypes and molecular mechanisms.

MECHANISTIC VALIDATION AND TOOLS FOR HIGH-CONTENT STUDIES

While planarians are not genetic model organisms like nematodes or zebrafish, there is a robust repertoire of cellular and molecular tools that allow for mechanistic studies of toxicity (Figure 3). Observations of tissue morphology have been popular readouts of planarian toxicity since the earliest planarian toxicology studies, especially during regeneration, because of their accessibility (Child, 1911; Hagstrom et al., 2016). The extent of head regeneration can be tracked by the reappearance of prominent head structures, such as the eyes and auricles, either by eye or using basic light microscopy. Notably, the auricles are not as prominent in all planarian species (Emmons-Bell et al., 2015) and thus not as broadly used as eye regeneration. While eye regeneration has historically been scored manually, machine learning algorithms have been created to predict the presence of eyes from high resolution videos of regenerating planarians (Zhang et al., 2019a).

The regenerative blastema that forms at the wound site after amputation (or asexual reproduction) has also been used as a measure of regeneration defects. The blastema is easily discernible from the pre-existing tissue because of its lack of pigmentation. As the planarian regenerates, the blastema grows and begins to reform the necessary anatomical structures. The rate of blastema growth can be quantified to identify regeneration defects or delays (Hagstrom et al., 2015, 2016; Kang et al., 2021).

In addition to these gross morphological readouts, effects on specific organs/cellular populations can be assessed using molecular biology techniques. For example, neuronal morphology can be observed via *in situ* hybridization (mRNA expression) or immunohistochemistry (protein expression) with pan-neuronal or subtype-specific neuronal markers (Ross et al., 2017). These allow for both qualitative observations of neuronal morphology and quantitative measures, such as of brain size (Hagstrom et al., 2015) or of abundance of certain neuronal subpopulations (Nishimura et al., 2011). Dynamic processes such as cell proliferation can also be quantified using standard techniques such as anti-phospho-Histone H3 antibody staining; while cellular damage including cell death or DNA damage can be assessed using terminal deoxynucleotide transferase dUTP nick end labeling (TUNEL) staining or the Comet assay, respectively, as for example done in (Majid et al., 2022).

Colorimetric assays can be used to quantify the activity of hydrolases, oxidases, and other enzymes in homogenates of exposed planarians during neurodevelopment or in adulthood. The activity of oxidative stress related enzymes, such as catalase and superoxide dismutase, has been a popular molecular biomarker of toxicity in planarians (Zhang et al., 2014, 2018). Additionally, the activity of neuronal enzymes, such as acetylcholinesterase (AChE), an important toxicological target (Russom et al., 2014), have been quantified (Ireland et al., 2022b; Hagstrom et al., 2018; Li, 2008). Ellman assays measuring AChE activity have been used to investigate whether behavioral phenotypes induced by exposure to organophosphorus pesticides were linked to AChE inhibition (Ireland et al., 2022b). It

was found that AChE inhibition was insufficient to explain the observed behavioral effects, in line with studies in other systems that suggest that NT resulting from chronic low-dose exposure to organophosphorus pesticides is independent of AChE inhibition (Costa, 2018; Voorhees et al., 2017). In addition to quantitative measures of enzymatic activity in homogenates, whole-mount staining of AChE activity has been used to qualitatively observe differences in AChE activity in the whole planarian (Hagstrom et al., 2018; Zhang et al., 2019b).

A more broadly applicable tool than biochemical assays, which are only available for certain enzymes, is quantitative PCR (qPCR). QPCR enables inexpensive and rapid studies of gene expression for candidate genes. For example, Balestrini et al. (2014) used quantitative RT-PCR to investigate the pathways of toxicity resulting from berberine exposure (Balestrini et al., 2014). A review of commonly assayed genes can be found in (Hagstrom et al., 2016). Importantly, the role of a candidate target can be further verified by knocking down the gene using RNAi (Shibata and Agata, 2018; Newmark et al., 2003). For example, the decreased heat sensitivity phenotype observed after exposure to the AChE inhibitors diazinon and physostigmine was recapitulated by double knockdown of the two identified cholinesterase genes in *D. japonica* (*Djche-1* and *Djche-2*), demonstrating that this phenotype is due to cholinesterase inhibition (Hagstrom et al., 2018).

CURRENT AND FUTURE CHALLENGES FOR PLANARIAN NT/DNT STUDIES

As a newcomer in modern toxicology, there remains much work to be done to develop planarian screening into a robust and standardized test method that could be integrated into a DNT test battery. As with all models, standardization of the best practices are essential, these include the species used (Ireland et al., 2020), planarian husbandry (planarian maintenance, water conditions (Child, 1930), circadian rhythm, and food (Zhang et al., 2019a)), chemical exposure conditions (static versus daily exchanges, temperature (Ding et al., 2019)), screening methodology, and data analysis. Transparency of research methods and data availability in public repositories which allow for meta-analyses are a critical aspect of this task. Conversations between the various stakeholders are essential to better understand what the current needs are and the opportunities that planarian toxicity testing could fill.

For integration into a test battery, the future of this system likely lies in being a first-tier rapid screening platform. With this in mind, current knowledge (Ireland et al., 2020; Blackiston et al., 2010) suggests that *D. japonica* is the best suited planarian species for this application. In addition, for chronic studies, only static exposure conditions are realistic for such high-throughput applications, else it is impossible to achieve the desired chemical coverage in a short period of time and at a reasonable cost. In terms of biological replicates, n=24 specimen were shown to suffice for robust screening results in 48-well plates on an automated platform (Zhang et al., 2019b). To integrate and compare results from planarian screening with data from other test methods, statistical analysis using benchmark concentrations or point of departure are indispensable. This approach has recently been implemented in a study of the NT and DNT of organophosphorus compounds in *D. japonica* (Ireland et al., 2022b).

It remains to be established what the context of use is for the planarian system, considering its unique strengths and limitations. We need to better understand the toxicokinetics and toxicodynamics of planarians, as only limited studies of planarian xenobiotic metabolism have been undertaken (Ireland et al., 2022a). How does planarian screening complement other models when integrated into a DNT test battery? How predictive is planarian testing of human toxicity?

CONCLUDING REMARKS

Freshwater planarians are an old model to study chemical effects on brain function and neuro-regeneration that have recently re-emerged as a promising organismal model for rapid NT/DNT screening. The development of standard protocols, as the examples provided in this special issue, is a first step to widen the field of researchers working together to establish this alternative model as a cost-effective and reliable NT/DNT model of the 21st century. Such a community effort will be indispensable for this task as it requires a monumental effort that cannot be achieved by individuals and will take time and perseverance.

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DATA AVAILABILITY STATEMENT

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LITERATURE CITED:

Bailey J, Thew M, and Balls M 2014. An analysis of the use of animal models in predicting human toxicology and drug safety. ATLA Alternatives to Laboratory Animals 42:181–199. Available at: https://journals.sagepub.com/doi/10.1177/026119291404200306?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed [Accessed October 6, 2022]. [PubMed: 25068930]

Bailey J, Thew M, and Balls M 2015. Predicting human drug toxicity and safety via animal tests: Can any one species predict drug toxicity in any Other, and do Monkeys Help? 10.1177/026119291504300607 43:393–403. Available at: https://journals.sagepub.com/doi/10.1177/026119291504300607 [Accessed October 6, 2022].

Balestrini L, Isolani ME, Pietra D, Borghini A, Bianucci AM, Deri P, and Batistoni R 2014. Berberine exposure triggers developmental effects on planarian regeneration. Scientific Reports 4:4914. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4014983&tool=pmcentrez&rendertype=abstract [Accessed October 5, 2015]. [PubMed: 24810466]

Bayingana K, Ireland D, Rosenthal E, Rabeler C, and Collins E-MS 2022. Adult and regenerating planarians respond differentially to chronic drug exposureo Title. bioRxiv:502372. Available at: https://www.biorxiv.org/content/10.1101/2022.08.02.502372v1.full.

Best JB, and Morita M 1982. Planarians as a model system for in vitro teratogenesis studies. Teratogenesis, carcinogenesis, and mutagenesis 2:277–91. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6130627. [PubMed: 6130627]

Blackiston D, Shomrat T, Nicolas CL, Granata C, and Levin M 2010. A second-generation device for automated training and quantitative behavior analyses of molecularly-tractable model organisms. PLoS ONE 5:1–20.

Boyd W, Smith M, and Freedman J 2012. Caenorhabditis elegans as a model in developmental toxicology. *In* Developmental Toxicology (Harris C and Hansen JM, eds.) pp. 15–24. Humana Press Available at: 10.1007/978-1-61779-867-2_3.

Brown DDR, Molinaro AM, and Pearson BJ 2018. The planarian TCF/LEF factor Smed-tcf1 is required for the regeneration of dorsal-lateral neuronal subtypes. Developmental Biology 433:374–383. [PubMed: 29291981]

Cebrià F, Kudome T, Nakazawa M, Mineta K, Ikeo K, Gojobori T, and Agata K 2002. The expression of neural-specific genes reveals the structural and molecular complexity of the planarian central nervous system. Mechanisms of Development 116:199–204. Available at: http://www.sciencedirect.com/science/article/pii/S092547730200134X [Accessed April 1, 2016]. [PubMed: 12128224]

Child CM 1911. Experimental control of morphogenesis in the regulation of Planaria. Biological Bulletin 20:309–331. Available at: http://www.jstor.org.

Child CM 1930. The susceptibility of Planaria to potassium cyanide in relation to hydrion concentration and to certain changes in salt content of the medium. Physiological Zoology 3:90–134. Available at: https://www.jstor.org/stable/30151087?seq=4#metadata_info_tab_contents [Accessed October 14, 2022].

Cochet-Escartin O, Mickolajczk KJ, and Collins E-MS 2015. Scrunching: a novel escape gait in planarians. Physical Biology 12:055001. Available at: http://iopscience.iop.org/article/10.1088/1478-3975/12/5/056010/meta;jsessionid=CED46064FD6A144301B89354A5E7B576.c2.iopscience.cld.iop.org. [PubMed: 26228740]

Costa LG 2018. Organophosphorus compounds at 80: Some old and new issues. Toxicological Sciences 162:24–35. [PubMed: 29228398]

Ding X, Song L, Han Y, Wang Y, Tang X, Cui G, and Xu Z 2019. Effects of Fe3+ on Acute Toxicity and Regeneration of Planarian (Dugesia japonica) at Different Temperatures. BioMed Research International 2019:1–9. Available at: /pmc/articles/PMC6724543/ [Accessed October 7, 2022].

Dong Z, Huo J, Liang A, Chen J, Chen G, and Liu D 2021. Gamma-Secretase Inhibitor (DAPT), a potential therapeutic target drug, caused neurotoxicity in planarian regeneration by inhibiting Notch signaling pathway. Science of the Total Environment 781:1–10.

Emmons-Bell M, Durant F, Hammelman J, Bessonov N, Volpert V, Morokuma J, Pinet K, Adams DS, Pietak A, Lobo D, et al. 2015. Gap Junctional Blockade Stochastically Induces Different Species-Specific Head Anatomies in Genetically Wild-Type Girardia dorotocephala Flatworms. International journal of molecular sciences 16:27865–27896. Available at: https://pubmed.ncbi.nlm.nih.gov/26610482/ [Accessed September 8, 2022]. [PubMed: 26610482]

Fraguas S, Barberán S, Ibarra B, Stöger L, and Cebrià F 2012. Regeneration of neuronal cell types in Schmidtea mediterranea: an immunohistochemical and expression study. The International journal of developmental biology 56:143–53. [PubMed: 22451002]

Fries EFB 1928. Drug action in galvantotropic responses. The Journal of general physiology 11:507–513. Available at: https://pubmed.ncbi.nlm.nih.gov/19872416/ [Accessed October 14, 2022]. [PubMed: 19872416]

Grebe E, and Schaeffer DJ 1991. Planarians in toxicology, standardization of a rapid neurobehavioral toxicity test using phenol in a crossover study. Bulletin of environmental contamination and toxicology 46:866–70. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1863793 [Accessed October 31, 2015]. [PubMed: 1863793]

Grohme MA, Schloissnig S, Rozanski A, Pippel M, Young GR, Winkler S, Brandl H, Henry I, Dahl A, Powell S, et al. 2018. The genome of Schmidtea mediterranea and the evolution of core cellular mechanisms. Nature 554:56–61. Available at: http://www.nature.com/articles/nature25473 [Accessed June 24, 2019]. [PubMed: 29364871]

Hagstrom D, Cochet-Escartin O, and Collins E-MS 2016. Planarian brain regeneration as a model system for developmental neurotoxicology. Regeneration 3:65–77. Available at: http://doi.wiley.com/10.1002/reg2.52 [Accessed March 21, 2016]. [PubMed: 27499880]

Hagstrom D, Cochet-Escartin O, Zhang S, Khuu C, and Collins E-MS 2015. Freshwater planarians as an alternative animal model for neurotoxicology. Toxicological Sciences 147:270–285. Available at: http://toxsci.oxfordjournals.org/content/early/2015/06/25/toxsci.kfv129.abstract?keytype=ref&ijkey=JVYmJ7DleP7igML [Accessed June 30, 2015]. [PubMed: 26116028]

Hagstrom D, Truong L, Zhang S, Tanguay R, and Collins E-MS 2019. Comparative analysis of zebrafish and planarian model systems for developmental neurotoxicity screens using an 87-compound library. Toxicological Sciences 167:15–25. Available at: https://academic.oup.com/toxsci/advance-article/doi/10.1093/toxsci/kfy180/5053695 [Accessed December 26, 2018]. [PubMed: 30011007]

Hagstrom D, Zhang S, Ho A, Tsai ES, Radić Z, Jahromi A, Kaj KJ, He Y, Taylor P, and Collins EMS 2018. Planarian cholinesterase: molecular and functional characterization of an evolutionarily ancient enzyme to study organophosphorus pesticide toxicity. Archives of Toxicology 92:1161–1176. Available at: http://link.springer.com/10.1007/s00204-017-2130-7 [Accessed December 18, 2017]. [PubMed: 29167930]

He JH, Gao JM, Huang CJ, and Li CQ 2014. Zebrafish models for assessing developmental and reproductive toxicity. Neurotoxicology and Teratology 42:35–42. [PubMed: 24503215]

Inoue T, and Agata K 2022. Quantification of planarian behaviors. Development, Growth & Differentiation 64:16–37. Available at: https://onlinelibrary.wiley.com/doi/full/10.1111/dgd.12765 [Accessed November 14, 2022].

Inoue T, Hoshino H, Yamashita T, Shimoyama S, Agata K, Agata K, Soejima Y, Kato K, Kobayashi C, Umesono Y, et al. 2015. Planarian shows decision-making behavior in response to multiple stimuli by integrative brain function. Zoological Letters 1:1–15. [PubMed: 26605046]

Inoue T, Kumamoto H, Okamoto K, Umesono Y, Sakai M, Sánchez Alvarado A, and Agata K 2004. Morphological and functional recovery of the planarian photosensing system during head regeneration. Zoological science 21:275–83. Available at: 10.2108/zsj.21.275 [Accessed November 3, 2015]. [PubMed: 15056922]

Inoue T, Yamashita T, and Agata K 2014. Thermosensory signaling by TRPM is processed by brain serotonergic neurons to produce planarian thermotaxis. The Journal of neuroscience : the official journal of the Society for Neuroscience 34:15701–14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25411498 [Accessed July 29, 2015]. [PubMed: 25411498]

Ireland D, Bochenek V, Chaiken D, Rabeler C, Onoe S, Soni A, and Collins E-MS 2020. Dugesia japonica is the best suited of three planarian species for high-throughput toxicology screening. Chemosphere 253:126718. [PubMed: 32298908]

Ireland D, Rabeler C, Gong T, and Collins E-MS 2022a. Bioactivation and detoxification of organophosphorus pesticides in freshwater planarians shares similarities with humans. Archives of Toxicology. Available at: https://link.springer.com/article/10.1007/s00204-022-03387-y#citeas.

Ireland D, Zhang S, Bochenek V, Hsieh J-H, Rabeler C, Meyer Z, and Collins E-MS 2022b. Differences in neurotoxic outcomes of organophosphorus pesticides revealed via multi-dimensional screening in adult and regenerating planarians. Frontiers in Toxicology 4:948455. Available at: https://www.frontiersin.org/articles/10.3389/ftox.2022.948455/full. [PubMed: 36267428]

Ivankovic M, Haneckova R, Thommen A, Grohme MA, Vila-Farré M, Werner S, and Rink JC 2019. Model systems for regeneration: Planarians. Development (Cambridge) 146. Available at: https://journals.biologists.com/dev/article/146/17/dev167684/222983/Model-systems-for-regeneration-planarians [Accessed July 25, 2022].

Kang J, Chen J, Dong Z, Chen G, and Liu D 2021. The negative effect of the PI3K inhibitor 3-methyladenine on planarian regeneration via the autophagy signalling pathway. Ecotoxicology 30:1941–1948. Available at: https://link.springer.com/article/10.1007/s10646-021-02439-w [Accessed September 8, 2022]. [PubMed: 34403000]

Kapu MM, and Schaeffer DJ 1991. Planarians in toxicology. Responses of asexual Dugesia dorotocephala to selected metals. Bulletin of environmental contamination and toxicology 47:302–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1912708 [Accessed November 3, 2015]. [PubMed: 1912708]

Li M-H 2008. Effects of nonionic and ionic surfactants on survival, oxidative stress, and cholinesterase activity of planarian. Chemosphere 70:1796–803. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17905407 [Accessed April 10, 2015]. [PubMed: 17905407]

Majid S, Van Belleghem F, Ploem JP, Wouters A, Blust R, and Smeets K 2022. Interactive toxicity of copper and cadmium in regenerating and adult planarians. Chemosphere 297.

Meigs L, Smirnmova L, Rovida C, Leist M, and Hartung T 2018. Animal testing and its alternatives – the most important omics is economics. ALTEX 35:275–305. Available at: https://www.altex.org/index.php/altex/article/view/1134/1131 [Accessed October 6, 2022]. [PubMed: 30008008]

Middleton AM, Reynolds J, Cable S, Baltazar MT, Li H, Bevan S, Carmichael PL, Dent MP, Hatherell S, Houghton J, et al. 2022. Are Non-animal Systemic Safety Assessments Protective? A Toolbox and Workflow. Toxicological Sciences 189:124–147. Available at: https://academic.oup.com/toxsci/article/189/1/124/6641985 [Accessed October 6, 2022]. [PubMed: 35822611]

Mineta K, Nakazawa M, Cebria F, Ikeo K, Agata K, and Gojobori T 2003. Origin and evolutionary process of the CNS elucidated by comparative genomics analysis of planarian ESTs. Proceedings of the National Academy of Sciences of the United States of America 100:7666–71. Available at: http://www.pnas.org/content/100/13/7666.full [Accessed November 16, 2014]. [PubMed: 12802012]

Morgan TH 1889. Experimental studies of the regeneration of Planarian maculata. Archiv für Entwickelungsmechanik der Organismen 7:364–397.

Newmark PA, Reddien PW, Cebrià F, and Alvarado AS 2003. Ingestion of bacterially expressed double-stranded RNA inhibits gene expression in planarians. Proceedings of the National Academy of Sciences of the United States of America 100:11861–11865. Available at: https://www.pnas.org/doi/abs/10.1073/pnas.1834205100 [Accessed November 8, 2022]. [PubMed: 12917490]

Nishimura K, Inoue T, Yoshimoto K, Taniguchi T, Kitamura Y, and Agata K 2011. Regeneration of dopaminergic neurons after 6-hydroxydopamine-induced lesion in planarian brain. Journal of Neurochemistry 119:1217–1231. [PubMed: 21985107]

Nishimura K, Kitamura Y, Umesono Y, Takeuchi K, Takata K, Taniguchi T, and Agata K 2008. Identification of glutamic acid decarboxylase gene and distribution of GABAergic nervous system in the planarian Dugesia japonica. Neuroscience 153:1103–14. [PubMed: 18440152]

Van Norman GA 2019. Limitations of Animal Studies for Predicting Toxicity in Clinical Trials: Is it Time to Rethink Our Current Approach? JACC: Basic to Translational Science 4:845. Available at: /pmc/articles/PMC6978558/ [Accessed May 24, 2022]. [PubMed: 31998852]

OECD 2021. Guideline No. 497 Guideline on Defined Approaches for Skin Sensitisation Section 4 Health effects. OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. Available at: http://www.oecd.org/termsandconditions/. [Accessed January 10, 2022].

Pagán OR, Coudron T, and Kaneria T 2009. The Flatworm Planaria as a Toxicology and Behavioral Pharmacology Animal Model in Undergraduate Research Experiences. Journal of Undergraduate Neuroscience Education 7:A48. Available at: /pmc/articles/PMC3592692/ [Accessed October 7, 2022]. [PubMed: 23493443]

Pearl R 1903. The movements and reactions of fresh-water planarians : a study in animal behavior. Quarterly Journal of microscopical science 4:509–714. Available at: https://openlibrary.org/books/OL23320755M/The_movements_and_reactions_of_fresh-water_planarians#details [Accessed August 29, 2022].

Pham LL, Watford SM, Pradeep P, Martin MT, Thomas RS, Judson RS, Setzer RW, and Paul Friedman K 2020. Variability in in vivo studies: Defining the upper limit of performance for predictions of systemic effect levels. Computational Toxicology 15:100126.

Raffa RB, Holland LJ, and Schulingkamp RJ 2001. Quantitative assessment of dopamine D2 antagonist activity using invertebrate (Planaria) locomotion as a functional endpoint. Journal of Pharmacological and Toxicological Methods 45:223–226. Available at: http://www.sciencedirect.com/science/article/pii/S1056871901001526 [Accessed November 3, 2015]. [PubMed: 11755386]

Robb SMC, Ross E, and Sánchez Alvarado A 2008. SmedGD: the Schmidtea mediterranea genome database. Nucleic acids research 36:D599–606. Available at: http://www.pubmedcentral.nih.gov/

articlerender.fcgi?artid=2238899&tool=pmcentrez&rendertype=abstract [Accessed November 16, 2014]. [PubMed: 17881371]

Ross KG, Currie KW, Pearson BJ, and Zayas RM 2017. Nervous system development and regeneration in freshwater planarians. Wiley Interdisciplinary Reviews: Developmental Biology 6:1–26.

Rovida C, Alépée N, Api AM, Basketter DA, Bois FY, Caloni F, Corsini E, Daneshian M, Eskes C, Ezendam J, et al. 2015. Integrated testing strategies (ITS) for safety assessment. Altex 32:25–40. [PubMed: 25413849]

Rozanski A, Moon H, Brandl H, Martín-Durán JM, Grohme MA, Hüttner K, Bartscherer K, Henry I, and Rink JC 2019. PlanMine 3.0—improvements to a mineable resource of flatworm biology and biodiversity. Nucleic Acids Research 47:D812–D820. Available at: https://academic.oup.com/nar/article/47/D1/D812/5212006 [Accessed June 4, 2019]. [PubMed: 30496475]

Russom CL, LaLone CA, Villeneuve DL, and Ankley GT 2014. Development of an adverse outcome pathway for acetylcholinesterase inhibition leading to acute mortality. Environmental toxicology and chemistry 33:2157–2169. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24922588. [PubMed: 24922588]

Sabry Z, Ho A, Ireland D, Rabeler C, Cochet-Escartin O, and Collins EMS 2019. Pharmacological or genetic targeting of Transient Receptor Potential (TRP) channels can disrupt the planarian escape response. PLoS ONE 14:e0226104. Available at: 10.1371/journal.pone.0226104. [PubMed: 31805147]

Sabry Z, Rabeler C, Ireland D, Bayingana K, and Collins EMS 2020. Planarian scrunching as a quantitative behavioral readout for noxious stimuli sensing. Journal of Visualized Experiments 2020:1–18. Available at: https://www.jove.com/v/61549/planarian-scrunching-as-quantitative-behavioral-readout-for-noxious [Accessed December 16, 2020].

Sabry Z, Wang R, Jahromi A, Rabeler C, Kristan WB, and Collins E-MS 2022. Head removal enhances planarian electrotaxis. Journal of Experimental Biology 225. Available at: https://journals.biologists.com/jeb/article/225/17/jeb243972/276550/Head-removal-enhances-planarian-electrotaxis [Accessed September 8, 2022].

Sachana M, Shafer TJ, and Terron A 2021. Toward a better testing paradigm for developmental neurotoxicity: OECD efforts and regulatory considerations. Biology 10:1–11.

Shen C, and Zuo Z 2020. Zebrafish (Danio rerio) as an excellent vertebrate model for the development, reproductive, cardiovascular, and neural and ocular development toxicity study of hazardous chemicals. Environmental Science and Pollution Research 27:43599–43614. Available at: https://link.springer.com/article/10.1007/s11356-020-10800-5 [Accessed October 17, 2022]. [PubMed: 32970263]

Shibata N, and Agata K 2018. RNA interference in planarians: Feeding and injection of synthetic dsRNA. Methods in Molecular Biology 1774:455–466. Available at: https://link.springer.com/protocol/10.1007/978-1-4939-7802-1_18 [Accessed October 4, 2022]. [PubMed: 29916171]

Stowell NC, Goel T, Shetty V, Noveral J, and Collins E-MS 2021. Quantifying Planarian Behavior as an Introduction to Object Tracking and Signal Processing. The Biophysicist 2:1–17. Available at: https://meridian.allenpress.com/the-biophysicist/article/2/3/1/464616/Quantifying-Planarian-Behavior-as-an-Introduction [Accessed August 29, 2022].

Talbot J, and Schötz E-M 2011. Quantitative characterization of planarian wild-type behavior as a platform for screening locomotion phenotypes. The Journal of experimental biology 214:1063–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21389189 [Accessed March 27, 2013]. [PubMed: 21389189]

Tejeda-Benitez L, and Olivero-Verbel J 2016. Caenorhabditis elegans, a biological model for research in toxicology. In Reviews of Environmental Contamination and Toxicology, Volume 237. (de Voogt W, ed.) pp. 1–35. Springer, Cham Available at: http://link.springer.com/10.1007/978-3-319-23573-8_1 [Accessed November 27, 2017]. [PubMed: 26613986]

Tian Q, Guo Q, Guo Y, Luo L, Kristiansen K, Han Z, Fang H, and Zhang S 2022. Whole-genome sequence of the planarian Dugesia japonica combining Illumina and PacBio data. Genomics 114:110293. [PubMed: 35139429]

Venturini G, Stocchi F, Margotta V, Ruggieri S, Bravi D, Bellantuono P, and Palladini G 1989. A pharmacological study of dopaminergic receptors in planaria. Neuropharmacology 28:1377–1382. [PubMed: 2559350]

Voorhees JR, Rohlman DS, Lein PJ, and Pieper AA 2017. Neurotoxicity in preclinical models of occupational exposure to organophosphorus compounds. Frontiers in Neuroscience 10:590. Available at: /pmc/articles/PMC5241311/ [Accessed March 8, 2022]. [PubMed: 28149268]

Werner S, Rink JC, Riedel-Kruse IH, and Friedrich BM 2014. Shape mode analysis exposes movement patterns in biology: Flagella and flatworms as case studies. PLOS ONE 9:e113083. [PubMed: 25426857]

Wu J-P, Chen H-C, and Li M-H 2012. Bioaccumulation and toxicodynamics of cadmium to freshwater planarian and the protective effect of N-acetylcysteine. Archives of environmental contamination and toxicology 63:220–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22481525 [Accessed October 31, 2015]. [PubMed: 22481525]

Wu JP, and Li MH 2018. The use of freshwater planarians in environmental toxicology studies: Advantages and potential. Ecotoxicology and Environmental Safety 161:45–56. [PubMed: 29859407]

Zhang HC, Ma KX, Yang YJ, Shi CY, Chen GW, and Liu DZ 2018. Molecular cloning, characterization, expression and enzyme activity of catalase from planarian Dugesia japonica in response to environmental pollutants. Ecotoxicology and Environmental Safety 165:88–95. [PubMed: 30193168]

Zhang S, Hagstrom D, Hayes P, Graham A, and Collins E-MS 2019a. Multi-behavioral endpoint testing of an 87-chemical compound library in freshwater planarians. Toxicological Sciences 167:26–44. Available at: https://academic.oup.com/toxsci/advance-article/doi/10.1093/toxsci/kfy145/5034903 [Accessed December 26, 2018]. [PubMed: 29893936]

Zhang S, Ireland D, Sipes NS, Behl M, and Collins E-MS 2019b. Screening for neurotoxic potential of 15 flame retardants using freshwater planarians. Neurotoxicology and teratology 73:54–66. Available at: https://www.sciencedirect.com/science/article/pii/S089203621830165X?via%3Dihub. [PubMed: 30943442]

Zhang X, Zhang B, Yi H, and Zhao B 2014. Mortality and antioxidant responses in the planarian (Dugesia japonica) after exposure to copper. Toxicology and industrial health 30:123–31. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22773437 [Accessed October 31, 2015]. [PubMed: 22773437]

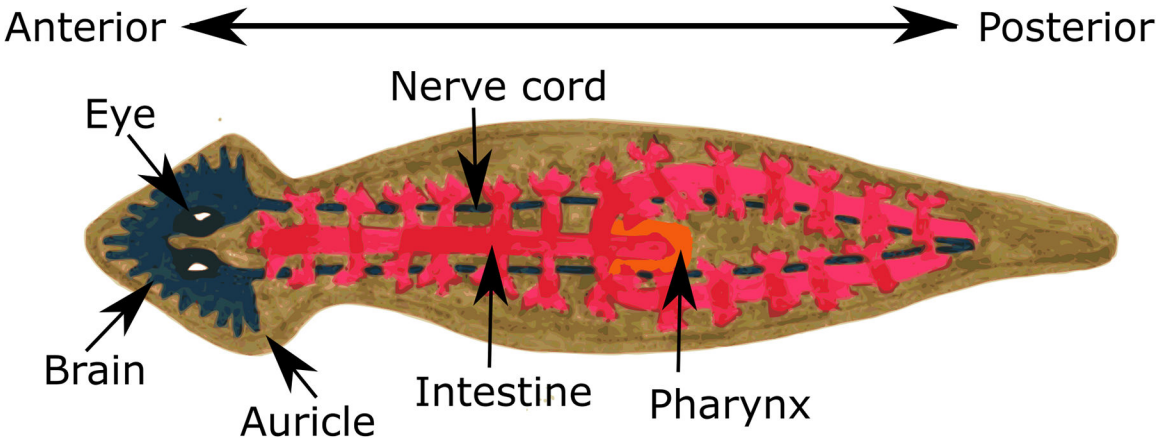

Figure 1. Freshwater planarian anatomy.

Planarians are soft-bodied, bilaterally symmetric flatworms with an anterior-posterior and dorsal-ventral body axes. Most of their body is taken up by a multi-branched intestine (red) that is connected to a pharynx (orange) which allows for food uptake and waste disposal. The planarian central nervous system (blue) consists of a bilobed spongy brain in the head and two ventral nerve cords that are interconnected by multiple commissures (commissures not shown). The eye spots are located on the dorsal side.

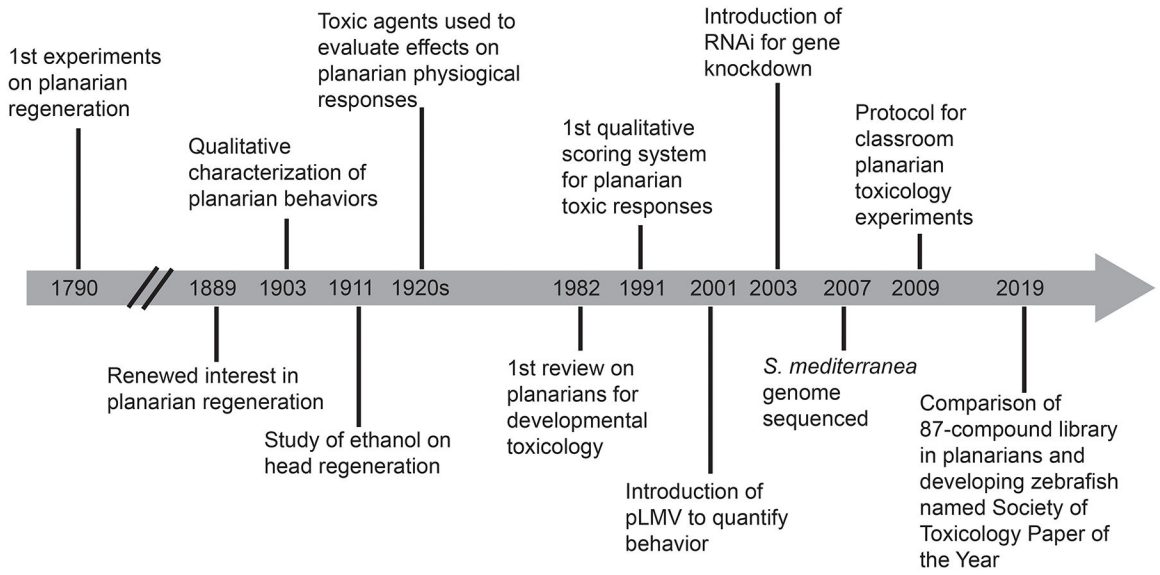

Figure 2. Timeline of key events in planarian toxicology research.

Experiments on planarian regeneration were first performed by G. Shaw in 1790 and later revisited by T. H. Morgan in 1889 (Morgan, 1889). The first qualitative assessment of planarian behavior in response to various stimuli was published by R. Pearl (Pearl, 1903). C.M. Child's thorough investigation of effectors of planarian regeneration included qualitative studies on the effects of ethanol (Child, 1911). Following this initial study, the 1920s saw a burst of planarian toxicology studies, which were largely focused on using toxic agents to better understand physiological responses, such as disintegration and certain behaviors, and how these were affected by different experimental parameters such as temperature or salt content in the water (e.g., (Child, 1930; Fries, 1928)). By 1982, the utility of planarians for developmental neurotoxicity and teratogenesis studies was recognized (Best and Morita, 1982). In 1991, Grebe and Schaeffer proposed the first qualitative scoring system to describe toxicity in planarians (Grebe and Schaeffer, 1991). In 2001, the planarian locomotor velocity (pLMV) method was introduced as a way to easily quantify differences in planarian behavior (Raffa et al., 2001). In the 2000s, the availability of a protocol for knocking down gene expression using RNA interference (RNAi) (Newmark et al., 2003) and the availability of the sequenced *Schmidtea mediterranea* genome (Robb et al., 2008) greatly advanced the planarian molecular biology toolkit. The growing use of planarians for toxicology research prompted the creation of a standardized protocol for experiments that could be conducted in undergraduate classrooms (Pagán et al., 2009). In 2019, the utility of using planarians for large-scale rapid screening was broadly recognized as a paper comparing the results from planarian and developing zebrafish screens on an 87-compound library was named the Society of Toxicology Paper of the Year (Hagstrom et al., 2019).

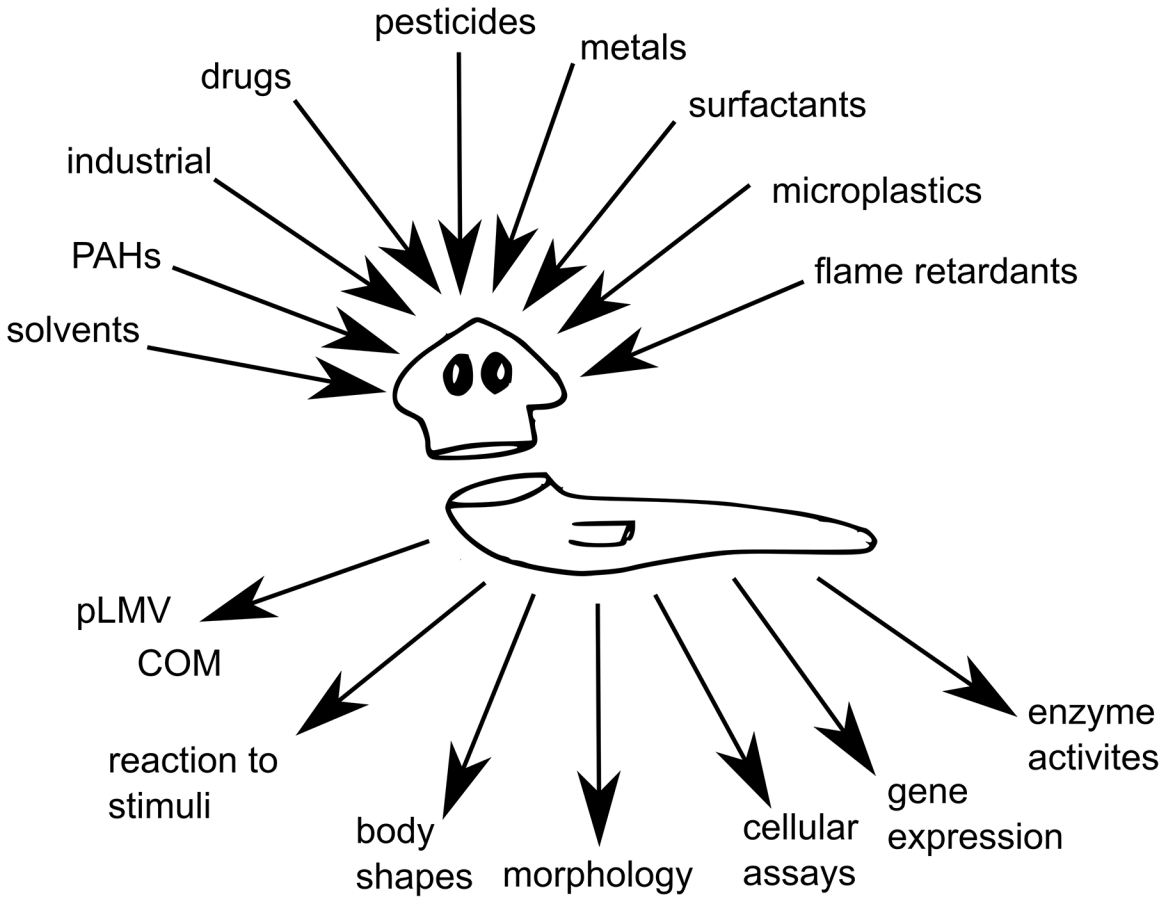

Figure 3. Overview of breadth of planarian toxicological studies.

A large variety of chemical agents (top) have been tested in planarians using different types of behavioral, morphological, cellular, and molecular readouts (bottom). These studies often utilize the planarian's ability to regenerate a new head, as indicated by the amputated planarian.