ANIMAL WELFARE REPORT 2017

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EXECUTIVE SUMMARY

Shell is committed to ending the need to do testing involving animals and strives to replace animal testing with suitable alternatives, while ensuring that we continue to innovate, develop and maintain safe new products and technologies and that we comply with regulatory requirements.

In 2017, research and development priorities included difficult to test substances (complex multi-constituent substances and/or substances with low water solubility), alternative test models for carcinogenicity, and non-vertebrate testing strategies to identify ecotoxic hazards of chemicals and effluents. Shell continued the development of innovative screening tests based on adverse outcome pathways for developmental and reproductive toxicity. Some of these screening tests have successfully been applied internally in our product development process. Test results are shared with regulators, with the aim of increasing their confidence in these tests.

The adverse outcome pathway approach is also used for the development of alternative testing strategies for ecotoxicology. The EcoToxChip programme is a multi-institutional and multi-sector collaborative research programme aiming to develop, test, validate and commercialise EcoToxChips, which cover key toxicity pathways of regulatory concern in three key vertebrate model species used globally in ecological risk assessment: fish, frog and bird. The participation in the development of EcoToxChips fits with Shell's aim to decrease the dependence on vertebrate testing for compliance.

Shell is also proactively seeking non-vertebrate (alternative) testing approaches to trial as potential replacements for traditional vertebrate methods currently used for whole effluent toxicity (WET) testing in North America.

By presenting our research at conferences and through publications in peer-reviewed journals, we are contributing to the growing momentum for global regulatory acceptance of these alternative methods. Where required by law, Shell has evaluated product safety using animals and, wherever possible, the outcomes of the animal tests have been used to validate non-animal alternative testing methods.

With regards to the Shell animal use numbers for 2017, regulatory compliance remains the main reason for animal testing, especially in chemical safety testing for the European Union (EU) chemical safety regulation (REACH), and effluent testing in the USA and Canada. Wherever possible, such regulatory compliance tests are done jointly with other companies that have to comply with the same regulatory requirements. This avoids unnecessary duplication of animal tests and thus minimises the overall use of animals. Furthermore, in such tests we also gain information to facilitate the design and acceptance of non-animal tests.

INTRODUCTION

There are strong ethical, scientific and business reasons to move away from animal testing as the means to demonstrate product safety. However, for the time being, we live in a strictly regulated environment where animal testing is still required to demonstrate the safety of Shell's processes and products.

The "3Rs" (Replacement, Reduction and Refinement) are now broadly accepted as the fundamental ethical framework within which animal research should be conducted. Replacement means the substitution for conscious living higher animals of insentient material; Reduction means reduction in the number of animals used to obtain information of given amount and precision; Refinement means any measure taken to decrease in the severity of procedures applied to those animals which still have to be used (or the provision of better housing and husbandry).

Shell implements the 3Rs principles in animal testing wherever possible while meeting legal obligations and protecting human life and the environment. Any Shell-owned or Shell-operated company must follow the company's animal testing standards when performing laboratory-based, regulation-required toxicology studies on animals, even in countries that have less stringent requirements. Under Shell's standards, animal testing remains the last resort and the use of non-animal tests to generate equivalent information is the first choice.

Replacement Reduction Refinement

At least twice every year, the External Animal Welfare Panel (the Panel) examines and comments on the implementation of Shell's animal testing requirements. The Panel works with Shell to ensure good practice in laboratories. It also advises on how Shell should optimise its engagement externally with the development and application of the 3Rs. The membership and terms of reference of the External Animal Welfare Panel are provided at the end of this report.

This report details Shell's ongoing efforts to replace, reduce and refine animal testing by progressing new and alternative testing methods, and by increasing the use of in vitro assays. The report also describes Shell's external engagement and advocacy for the use of alternatives to traditional animal experimental methods. An overview of animal use by Shell to assess the safety characteristics and environmental impact of its products, operations and manufacturing processes are set out at the end of this report. This report has been reviewed and approved by the Panel.

3R ACTIVITIES FOR ANIMAL WELFARE

Regulatory compliance remains the main driver for animal use in Shell. The approach to animal welfare can be described by four main activities that support the principles of 3Rs (i.e., Reduction, Refinement and Replacement). Each activity notes a set of behaviours and mindset that guide Shell subject matter experts on animal welfare with the view of creating and practicing a culture of care. Priorities are selected based on their relevance to Shell's human and environmental safety assessment responsibilities. In addition, focus is given to overcome barriers to the progression of the 3Rs of animal tests. The four main activities are:

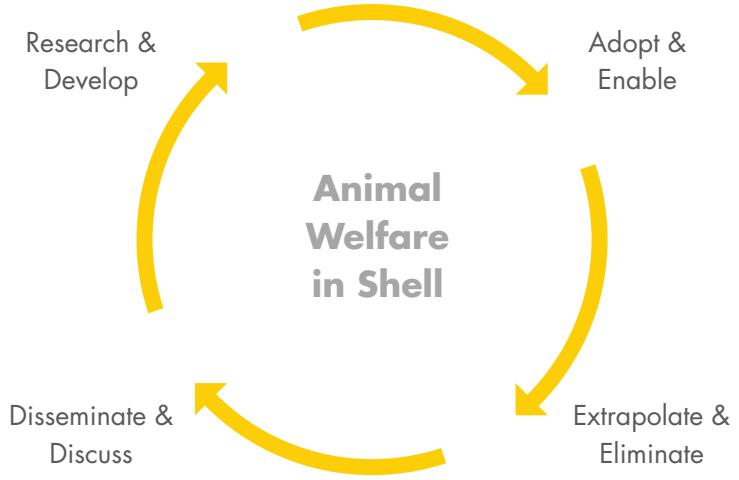
Research and develop are efforts related to collaboration, funding and conducting research for innovative hazard and exposure assessment methods. Drivers for prioritisation are business needs, and areas where the highest impact on the 3Rs can be achieved.

Adopt and enable aims to adopt our research advances, learnings, and external good practice into Shell's practices. Shell implements the advancements and insights into internal hazard and exposure assessment activities. In addition, by promoting a culture of care in industry organisations where Shell is active, we can identify and enable best practice for animal welfare and reduce animal testing in product safety and regulatory compliance.

Extrapolate and eliminate focuses on collaboration to minimising or eliminating animal use by leveraging existing data and prediction models. Integration of information from multiple sources can be achieved by establishing, utilising and maintaining access to databases. Internally gained insights are extrapolated to external applications to build confidence in the innovative methods. Collaboration with external parties for this activity is essential.

Disseminate and discuss includes publishing of results, presenting data and ideas in professional fora, engaging with regulators and academic circles. It also includes the teaching of good practice, and review of acquired knowledge by peers, as well as with an external panel. This approach aims to instil a culture of care at the highest scientific and practical level. It also intends to achieve a wide acceptance of insights and to generate new ideas that feed back into the activity circles.

The following sections of this report highlight Shell's efforts and progress in each of these activities



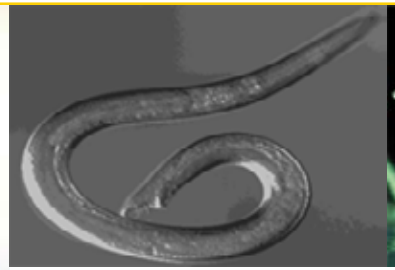
RESEARCH AND DEVELOP

Covers Shell's research and other efforts related to collaboration, funding and conducting research for innovative hazard and exposure assessment methods. Drivers for prioritisation are business needs, and areas where the highest impact on the 3Rs can be achieved.

For 2017, research and development priorities included difficult to test substances (complex multi-constituent substances and/or substances with low water solubility), alternative testing strategies to identify human developmental and reproductive hazards, test models for carcinogenicity, modelling approaches, and non-vertebrate testing strategies to identify ecotoxic hazards of chemicals and effluents.

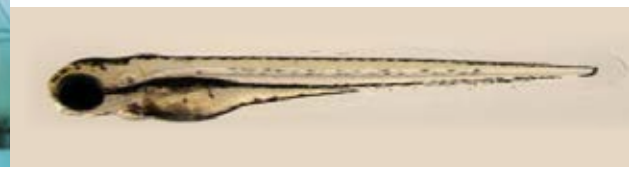
Alternative testing strategies to identify human developmental and reproductive toxicity (DART)

Identification or prediction of potential hazards to reproductive organs or the developing embryo (developmental and reproductive toxicity – DART) is complex, since DART often depends on an interplay between different organs and delicate hormonal balances. Shell has been involved in a calibration study that combines the use of a microscopic worm – nematode (*Caenorhabditis elegans*) with zebrafish embryos (*Danio rerio*) as alternative organisms to assess chemicals for DART effects.



C. elegans

D. discoidium



D. rerio

Candidate chemicals tested in this screening battery show alignment with data obtained from conventional mammalian toxicity studies, indicating that these organisms have the potential to be used as DART screening systems (Racz, 2017; Rooseboom, 2017a; 2017c).

Shell is co-sponsoring the UK National Centre for 3Rs (NC3Rs) Challenge "DARTpaths" (https://crackit.org.uk/challenge-26-dartpaths). This Challenge builds upon previous work of the PREDART Challenge, where alternative non-mammalian model organisms (e.g. zebrafish, *C. elegans, D. discoidium*) were used to assess the potential effects of new chemicals on adult fertility and sexual behaviour, embryo implantation and the development of the foetus (Rooseboom et al., 2017b). The key assumption is that the non-mammalian model organisms share mechanisms and pathways related to developmental and reproductive toxicity and that responses measured at the molecular level can be interpreted as an expected effect in humans. The key to unlocking the potential of these alternative test systems is to understand the

commonality of biological pathways across different species. Therefore, the DARTpaths Challenge aims to integrate available information on the relationship between specific genes and specific physiology, or specific compounds and specific effects, of model organisms that include human, mouse, rat, rabbit, zebrafish, fruit fly, nematode and D. discoideum, so that gene-to-physiology or compound-effect relations between these organisms can be mapped. This map or framework will be used to translate data from test model organisms to predicted developmental toxicity in humans. The "DARTpaths Challenge" started in 2017 and is expected to be completed in 2020.

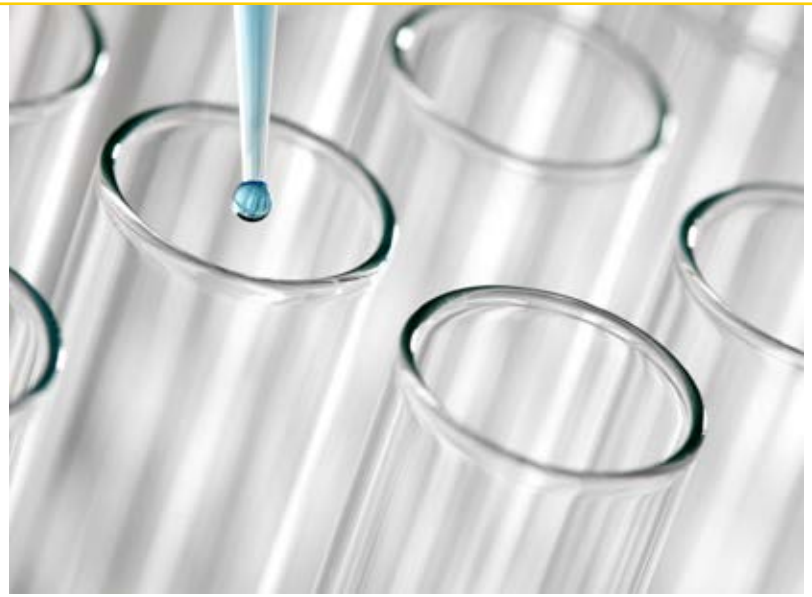
Based on historical data generated in animals it is hypothesised that polycyclic aromatic compounds (PAC) may cause developmental toxicity in rodents. Following on from work in previous years, Shell is involved in the development of screening tests which are designed to test this hypothesis. The potency of PAC rich substances was compared to PAC-free substances using a mouse embryonic stem cell test (EST). It was observed that the potency of the tested substances causing effects in the EST was directly proportional to their PAC content, which is in correlation with what is known from animal data in the open literature. The results validate the in-vitro screening method and strengthen the hypothesis that PAC may be inducers of embryonic developmental toxicity (Kamelia, 2017a; 2017b; Boogaard, 2017).

Screening model for carcinogenicity

For carcinogenicity, it has been demonstrated that the three- to seven-ring PAC are potentially hazardous molecules. Based on this premise, innovative screening methods may expand the applicability domain of the currently available standard tests. The ToxTracker assay which consists of a panel of mammalian stem cell lines that through fluorescence signal for DNA damage, oxidative stress and protein damage, can be applied to UVCB substances (unknown or variable composition, complex reaction products and biological materials) as a high-content screen assay by directly assessing the PAC activity . UVCB extracts with a high three- to seven-ring PAC content activated the genotoxicity reporters in the ToxTracker assay in line with the expected mode of action.
Because it was well correlated to existing industry screening methods, it is a promising additional tool to assess PAC-mediated carcinogenicity of UVCB substances (Hendriks, 2017).

Difficult to test substances

Most non-mammalian hazard assessment models use water-based systems. By nature, petroleum products are difficult to test due to their complex composition and low water solubility.
Shell is co-sponsoring the NC3Rs DoCE Challenge, which is intended to establish improved and increased throughput methods and approaches to better account for bioavailability through development of dosing and measurement strategies of test chemicals in in vitro assays (https://crackit.org.uk/challenge-27-doce).
Successful completion of this Challenge will deliver new capability (dosing and measurement of chemicals in vitro) to ensure concentration-response relationships determined from a range of in vitro test systems, that are reflective of human and environmental species in vivo exposure conditions. Improving confidence in the relevance of in vitro-derived data, through better understanding and control of exposure parameters, will deliver the reduction and replacement of animal testing. The DoCE Challenge has started in 2017 and is expected to be completed in 2020.



Modelling approaches

In silico models can be used to reduce or replace animal testing, and Shell has (co)developed several models for the prediction of e.g. aquatic toxicity and skin irritation (Shaigara 2017). Currently, Shell is sponsoring the NC3Rs RespiraTox Challenge, which is aimed at developing a quantitative structure activity relationships (QSARs)-based tool that reliably predicts human respiratory irritancy potential of chemicals (https://crackit.org.uk/challenge-28-respiratox).

The tool should fulfill the Organisation for Economic Co-operation and Development (OECD) principles for QSAR validation to demonstrate the statistical and mechanistic reliability of the model. This will endorse the model's use under regulatory context (e.g. REACH, Environmental Protection Agency (EPA)). The RespiraTox Challenge started in 2017 and is expected to be completed in 2018.

Non-vertebrate testing strategies to identify ecotoxic hazards of chemicals and effluents

EcoToxChip

The EcoToxChip programme is a multi-institutional and multi-sector collaborative research programme aiming to develop, test, validate and commercialise EcoToxChips (based on quantitative polymerase chain reaction arrays), which will consist of over 300 genes covering key toxicity pathways of regulatory concern in three key vertebrate model species used globally in ecological risk assessment (fish, frog and bird). EcoToxChips will also eventually be developed for three native species of fish, frog and bird of recreational and aboriginal concern in North America. A data evaluation tool (EcoToxXplorer.ca) will also be developed to allow end users to upload EcoToxChip data and interpret their results for the characterisation, prioritisation and management of environmental chemicals and complex mixtures of regulatory concern. Shell has been an active industrial collaborator on the project, providing advice to the research team on end-user needs for the EcoToxChip since it was awarded to McGill University and University of Saskatchewan by Genome Canada and Genome Quebec in 2016. To date, several standard chemicals representing a variety of chemical categories and physical-chemical properties have been

tested and evaluated, and the organisational framework of genes have been derived to design the EcoToxChip. In the future, Shell plans on trialling the prototype chips with our products and a model effluent as an additional contribution to the development process. The EcoToxChip has the potential to save a significant number of vertebrate organisms. Furthermore, it will help fill a major void of ecotoxicity data for key vertebrate taxa (i.e., frogs and birds) which is often unavailable or absent for many chemicals.

Whole effluent toxicity testing: Is there a non-vertebrate approach?

To decrease the dependence on vertebrate testing for compliance, Shell is proactively seeking non-vertebrate (alternative) testing approaches to trial as potential replacements for traditional vertebrate methods currently used for whole effluent toxicity (WET) testing in North America. To identify potential alternative methods, an extensive literature review was undertaken to identify and prioritise potential non-vertebrate alternative methods (Kristofco and Hughes, 2017). This selection process identified non-vertebrate bioassays applied regionally and elucidated alternative approaches that are already in use internationally for whole effluent assessment. The toxicity testing methods evaluated were selected from standardised methods. These were further refined based on their applicability to effluent testing in the North American compliance framework. Priority was given to those methods that were available commercially in North America, and included apical endpoints in methods similar in duration and style to current compliance testing methodologies. Additional priority was given to methods which had the greatest similarity to fish. An initial list of 64 standard protocols from agencies from around the world were compiled and refined

to a list of seven that reflected five testing frameworks for further performance testing. The advantages and challenges associated with each method were investigated. The output of this literature review is to eventually be used to design and perform research that compares the selected methods side-by-side with standard WET methods. It may be that no single method or test by itself is sufficient to replace vertebrate testing, but using a weight of evidence approach should help to reduce vertebrate testing.

ADOPT AND ENABLE

Covers application of our research and develop advances, learnings and good practice by others into Shell's practice. Shell implements the advancements and insights into internal hazard and exposure assessment activities. In addition, by promoting a culture of care in industry organisations where Shell is active, we can identify and enable good practice for 3Rs to reduce animal testing in product safety and regulatory compliance.

Relevance of animal models for human hazard assessment

Use of human cells?

With the aim of improved chemical safety testing, with better predictability of human safety, there is an increased focus on development of test models using human cells and tissues. Existing animal models have been used for decades to predict chemical safety for humans. However, these animal models are only models and have their inherent uncertainties when predicting the human situation. The challenge is to validate the alternative test systems based on human cells and tissues as animal models are not the most appropriate basis for this.

Choice of animal species?

When using mammalian models for human hazard assessment, a key question is which species (e.g. mice, rats or rabbits) is a better predictor for human effects. The answer is not always straightforward because, depending on the chemical and route of exposure, one species will be a better model than another one. This is a fundamental aspect in the development of 3R models as animal data is often used as a benchmark for validation. Tests on a chemical substance to determine the potential to induce developmental toxicity in the foetus through maternal exposure have indicated that selection of the species is important for the relevance of the results.

To answer the question of which animal model is more relevant for humans, further research has been conducted at industry level (where Shell is a member of a consortium), applying both in vitro techniques and in vivo tests using rats and rabbits. Explorative in vitro work has indicated that: 1) the toxicity of this compound is caused by its metabolites; 2) the toxic metabolites can be transported through placenta from the maternal side to the foetuses in rodents, but not in rabbits; and 3) regarding the transportation mechanism of these toxic metabolites, rabbit placenta is a better match to human placenta. Based on in vitro findings, in vivo work can be refined and focused. In 2017, the rat experiments were completed, while the rabbit experiments are planned for 2018.

Insights gained from these experiments, on the mode of action and kinetics, will be used to help the development of future testing strategies to predict human developmental toxicity.

Adaptation of animal models for human relevance

A high production volume chemical is the building block for several industrial and household products. When inhaled this chemical has caused lung cancer in mice but not in rats. This has led to two questions:

1 Are lung tumours seen in mice relevant to humans?

2 What is the underlying mechanism resulting in these species differences and how does it relate to human risk.

A series of in vitro studies investigating the early stages of this substance's carcinogenic potential remained inconclusive in explaining the mode of action. Subsequent mode of action studies in mice demonstrated that the carcinogenic effect was caused by specific genes in the mice, and not by the human equivalent genes. Because in vitro studies on early stages of carcinogenicity have been shown not to reflect the in vivo situation; special studies with mice are necessary to answer the questions above.

To answer the second question of the underlying mechanism causing the differences in toxicity between mice and humans, a mouse study was performed. It was found that lung cancer in mice is initiated in the following chain of events; 1) metabolism of the chemical mediated by a mouse-specific gene in mice lungs; 2) consequent changes in levels of metabolism of lipids and lipoproteins in lungs, 3) resultant cell toxicity and cell-cycle disturbance. Altogether, these data support the finding that toxicity in the mouse is due to conversion of the inactive parent chemical to a toxic metabolite by a mouse-specific genetic mechanism that is neither quantitatively

or qualitatively relevant to the human. Thus, exposure to vapours of this chemical in occupation or household is not expected to increase the risk of lung cancer in humans (Andersen, 2017a, 2017b; Cruzan, 2017a, 2017b).

Not only species but also strain differences for human relevance

Rat oral studies are currently the default test approach to assess dietary exposure to chemicals. Conventionally the most sensitive relevant strain is used to extrapolate results to humans. However, depending of the chemical and route of exposure, one species or strain will be a better model over another one resulting in the question of relevance over sensitivity. An example is the Fisher (F344) rat, which develops effects that are never seen in other rat strains or humans. A critical review of the data will lead future research needs, that may focus on more human relevant endpoints (Carrillo, 2017; Fleming, 2017).

EXTRAPOLATE AND ELIMINATE

The extrapolate and eliminate activities focus on collaboration to minimising or eliminating the use of tests involving animals by the leveraging of data, for example through replication of learnings and successes across regulatory frameworks. Internally gained insights are extrapolated to external applications to build confidence in the innovative methods. Collaboration with external parties is essential for this. Wherever possible, such regulatory compliance tests are combined with those required of other companies. This minimises the use of animals not only for Shell but also members of the consortia. Furthermore, in such tests we seek to glean information that will identify biochemical changes and adverse outcome pathway information to facilitate the design and acceptance of non-animal tests.

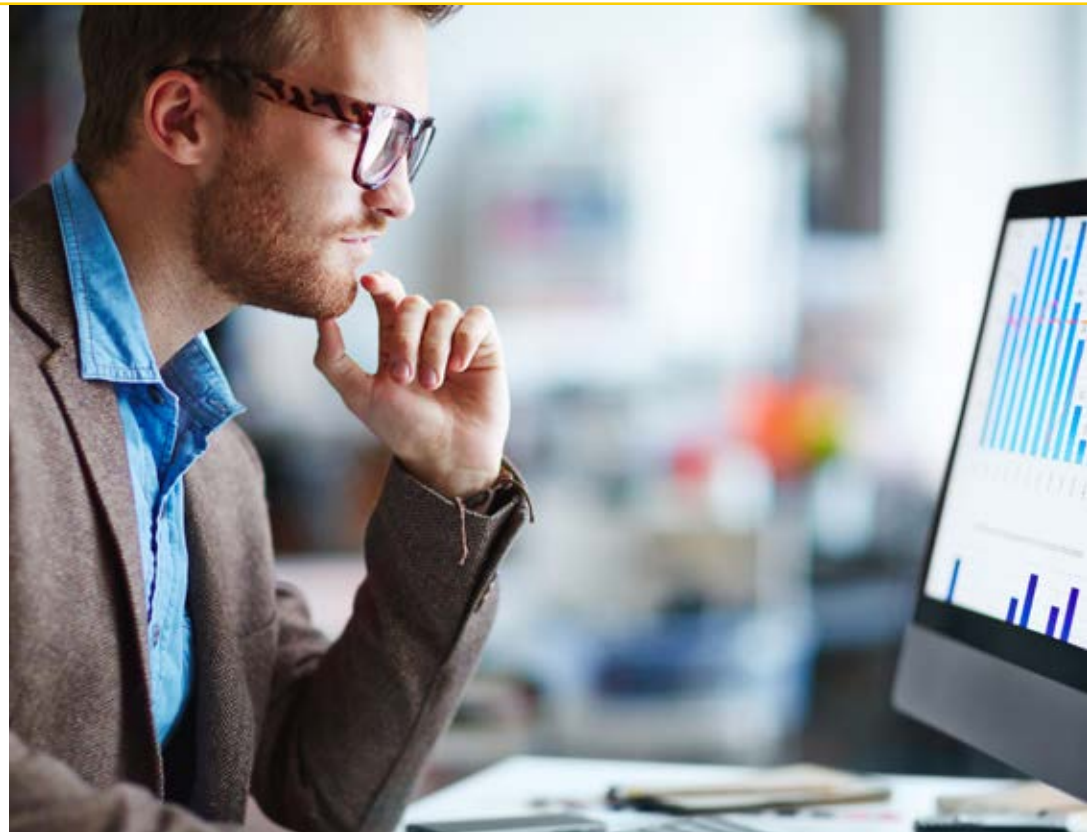
Extrapolate data and reduce or remove the need for animal testing: read-across in practice

The term "read-across" is used to refer to the grouping of similar chemical substances into "families" for chemical safety assessment based on the hypothesis that their similarity (functionality and physico-chemical properties) results in similar toxicity or a predictable trend in toxicity. Hence, toxicological properties of a data-rich family member can be "read-across" to data-poor family members without having to repeat the same animal studies. Solid read-across and grouping approaches enable reductions in animal testing, while maintaining product safety. This is particularly important for gas to liquid substances (GTL) which are substances of unknown or variable composition, complex reaction products and biological materials (UVCBs).

Currently, petroleum-derived and GTL UVCB substances are grouped based on refining history, because their refining or manufacturing history will drive the presence of specific types of molecules with known toxicity profiles. In 2016, Shell started a joint industry project investigating grouping of petroleum-based UVCBs, focusing on defining biological similarity between substances. This research, performed through the European refining industry environmental science organisation called CONCAWE, is conducted in collaboration with Texas A&M University. The most recent results demonstrate that petroleum substances can be grouped based on biological as well as analytical chemistry profiling (Grimm et al., 2017). Additional project details can be found on the CONCAWE website: (https://www.concawe.eu/mediaroom/cat-app-project/)

Commonalities and differences in toxicity and modes of action

Some chemicals have been in use and investigated for decades, resulting in a significant amount of toxicological data generated. Because this information may be scattered and not easily accessible a good industry practice is to gather, summarise and publish decades worth of literature. Shell actively participates in industry initiatives that review toxicological commonalities and differences in toxicity and modes of action. This results in good read-across and serves as a benchmark to develop 3R methods (Fowles, 2017).



Development of an ecological threshold of toxicological concern

In 2017, Shell was active in the ILSI-HESI (International Life Sciences Institute and Health and Environmental Sciences Institute) technical committee for alternatives for ecological risk assessment through a project to develop an ecological threshold of toxicological concern (or eco-TTC) database and web-based tool. The TTC concept is a well-established risk assessment tool for determining a human exposure concentration with negligible risk in the absence of chemical-specific data. The technical committee proposed an extension to the human safety TTC concept for application in environmental situations. eco-TTCs summarise the wealth of ecotoxicological information as predicted no-observed effect concentrations (PNECs) on diverse chemical substances in the form of statistical (probability) distributions. Eco-TTCs can be developed that allow prediction of untested chemicals based on structural attribute (category), mode of action, or functional use. The eco-TTC has been proposed to be a PNEC for ecological communities and establishes a concentration expected to have a de minimis probability that effects would be observed for a given group of compounds. The approach may be useful for assessing chemicals at early tiers of the risk assessment process, providing hazard perspective on chemicals that lack QSARs, guiding product development discussions, and assisting read-across or category justifications. The eco-TTC approach has the potential to reduce the need for vertebrate testing (e.g., fish) in many situations. Shell participated on the organising committee for an international workshop held in Ottawa, Canada, in September 2017 on the eco-TTC with the primary objective to discuss and evaluate the feasibility of the eco-TTC approach and evaluation of the eco-TTC database and web-based tools. Workshop participants made several observations and interpretations that led to refinements of the eco-TTC tool and helped lay the conceptual and scientific foundation necessary to apply eco-TTCs in risk assessment.

DISSEMINATE AND DISCUSS

Shell publishes animal numbers, results from (non)animal testing, and new approaches developed either independently or within a consortium to improve transparency and share data and good practices. Shell publishes in peer-reviewed journals, presents data and ideas in professional fora, and engages with regulators and academia. The overall goal is to instil a culture of care at the highest scientific and practical levels.

Exposing new ways of thinking

A key element necessary to transition towards utilising both non-animal and refined safety testing is the better understanding of chemical exposure. This includes measuring chemical concentrations directly in cell culture assays that enable simulation of real-life human exposures in these in vitro systems. Such approaches promise to increase the human relevance of safety assessment, and shift the focus from hazard to risk-driven strategies. Human exposure-based safety assessment offers scientific and 3Rs benefits across all sectors marketing chemical or medicinal products. Shell actively discusses this approach through the UK's National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) at expert working groups of scientists across the agrochemical, industrial chemical and pharmaceutical industries (Sewell, 2017).

Case studies and guidance of how to leverage exposure information and good practice for REACH compliance was the focus in 2017 (McKee, 2017a; 2017b). Exposure data may require the use of advanced statistical analysis to interpret complex data sets of human toxicity and environmental exposure, which is an area that requires much skill and expertise from regulators and industry (Cox, 2017; Boogaard, 2017).

Promote a culture of care

To act on our goal to instil a culture of care e.g. among our scientific peers, in 2017 we presented our research activities at international level where we focused on the following topics.

We were actively involved in three different regional meetings for the Society of Environmental Toxicology & Chemistry (SETAC) including Europe (Hughes et al., 2017a; Lagadic et al., 2017; Leonards et al., 2017a; 2017b), North America (Galus et al., 2017; Kilgour et al., 2017; Kristofco and Hughes, 2017; Lyon et al., 2017b; Meyer et al., 2017; Philibert et al., 2017; Redman et al., 2017), and, for the first time, Latin America (Lyon et al., 2017c; Whale et al., 2017). We also participated in the 10th Congress on Alternatives and Animal Use in the Life Sciences held Aug 25-30 in Seattle, Washington. We were invited to speak in the session on "New methods and novel approaches for assessing and monitoring environmental contaminant mixtures or individual priority substances" at the 44th Canadian Ecotoxicity Workshop (Oct 2-4,

Guelph, Ontario, Canada) where we presented on our literature review performed in 2017 to develop a shortlist of five potential candidate non-vertebrate alternative bioassays or assessment approaches to trial for whole effluent toxicity testing (Hughes and Kristofco, 2017c). The same talk was also presented again at the North American SETAC meeting in a session entitled "Whole effluent toxicity testing: a science evolving" (Kristofco and Hughes, 2017) and at a supplemental session at SETAC organised by the US EPA and the Humane Society entitled "Improving species extrapolation for protecting endangered species: what is available and what Is needed?". All these presentations were given with an intent to: 1) gain interest on the topic of non-vertebrate methods for WET testing of effluents; 2) seek collaborators for a research study trialling an alternative method for WET testing; and 3) get input from the regulatory community on the process to having alternative methods accepted for use in permit testing (for more detail see Research and develop section above).

Our work on mammalian toxicology was presented at the 3rd International Conference on Toxicity Testing Alternatives & Translational Toxicology in Nanjing China, where adaptations for difficult to test substances in direct peptide reactivity assay skin allergy tests was presented (Carrillo 2017). Also, the DART alternative test battery was not only discussed at this meeting (Rooseboom 2017), but also in London at the NC3R launch event and at the Reproductive and Development Toxicology Webinar Series, AICM subcommittee Toxicology, Ecotoxicology & Risk Assessment (TERA).

A poster presentation on a combinatorial model organism strategy to predict developmental and reproductive toxicology (DART) was presented at EUROTOX meeting, Bratislava, Slovak Republic (Rooseboom 2017). At the same meeting, the relevance of the F344 rat for risk assessment was disseminated (Carrillo 2017).

SHELL USE OF ANIMALS FOR TESTING IN 2017

In line with standard industry practices, Shell reports on the activities of Shell-owned and Shell-operated companies. Testing programmes that are supervised by industry consortia in which Shell or Shell joint ventures (JVs) participate are reported separately. Shell reports all experimental animal use on a 100%-basis (each animal is reported in Shell's figures, even if the testing programme is undertaken by multiple companies). Testing data is collected from internal sources and from reports provided by external testing laboratories.

Table 1: number of laboratory animals used, 2013 – 2017

Animal used	Tests commissioned	Number of animals per year				
		2013	2014	2015	2016	2017
Fish	Shell	44,696	61,773	76,476	42,926	32,732
Fish	Industry consortia	5,576	0	2,720	2,285	0
Fish	Joint ventures	10,020	20,720	6,260	10,140	1,920
Amphibians	Shell	0	0	5,770	12,180	17
Rodents	Shell	4,368	2,591	72	0	0
Rodents	Industry consortia	5,763	3,202	9,908	767	1,787
Rodents	Joint ventures	0	0	0	0	0
Rabbits	Shell	870	40	3	0	0
Rabbits	Industry consortia	4	0	20	24	3
Rabbits	Joint ventures	0	0	0	0	0
	TOTALS	**71,297**	**88,326**	**101,229**	**68,322**	**36,459**

Explanatory notes:

Industry consortia are groups of companies (including Shell) that co-operate, usually within the framework of an industry trade association, to share available data and the costs of testing programmes on particular chemicals or groups of chemicals.

Joint ventures include JVs where Shell has operational control. In instances where work was placed for a JV through an industry consortium, the data is reported under industry consortia.

The total number of laboratory animals used in procedures from 2013-2017 is shown in Table 1. For 2017, the total number of vertebrates (including mammalian, fish and amphibian species) is 36,459. This total is about half of the number reported in 2016 and is because Shell sold off its oil sands operations which represented a significant portion of regulatory required (permit) effluent testing numbers, as well as oil sands R&D work that was carried out over the past four years. In 2017, the use of fish for regulatory mandated effluent testing in North America remained the most significant contributor to the total number of animals used by Shell at 94% of total vertebrates.

For the third and final year, adult frogs and frog tadpoles were used as part of a three-year research programme for environmental studies to investigate the impact of oil sands operations on amphibians. However, the numbers reported in 2017 are significantly reduced as most work was performed on frog embryos in 2017, which are not considered a vertebrate. 2017 was the last year of the programme and results are in progress.

In 2017, all mammalian testing was carried out through industry consortia. The benefit of performing animal testing through consortia is that following agreed study designs avoids duplication of tests. Although Shell reports animal numbers on a 100%-basis, the specific impact of working through consortia over Shell's total animal numbers is shown in Table 2.
If the number of animals used in a consortium study is divided by the total number of consortium partners, a relative 'Shell share' of the total number of animals used is obtained. The calculation shows that from a total of 1,790 mammals used in consortia, the 'Shell share' was approximately 52 mammals. This clearly demonstrates the impact of working in consortia on the reduction of animal numbers.

Table 2 Mammalian species used for testing

Species	Total number	Number used in consortia	'Shell share' of animals used in consortia
Rats	1,650	1,650	41
Mice	137	137	10
Rabbits	3	3	1
TOTAL	**1,790**	**1,790**	**52**

Rabbits are used for REACH compliance where the in vitro test (OECD 437 bovine corneal opacity and permeability - BCOP) was indecisive. Mice were used for research on new models for skin sensitisation and for mode-of-action research. Rats were used for REACH compliance and research on mode of action.

Purpose of testing on animals in 2017

In the past years, Shell has indicated the purpose for animal testing using the categories 'product stewardship' and 'regulatory compliance'. Whereas the purpose of 'regulatory compliance' is self-explanatory, the purpose 'product stewardship' required further explanation. It was defined as data that is required to understand the health and environmental hazards of a product and not collected for regulatory purposes. This may include generation of detailed information on the mechanism of toxic action. This mechanism of action can inform the relevance of the used animal model for human risk assessment.

As Shell is using the 3Rs concepts to promote animal welfare, smart and combinatorial testing strategies are applied. For example, when obliged to conduct an animal test for regulatory compliance, there might be an opportunity to combine the mandated test with a research project which would maximise the use of information obtained from the used animals. This research project would typically generate data to advance 3R methodologies or enhance the information of Shell's chemical portfolio. These initiatives were collectively described as 'product stewardship' in previous reports, but as in reality the testing has been used for research purposes and with the 3Rs in mind, from 2017 onwards Shell will use the collective term of '3Rs and research'.

As seen in Figure 1, since 2010, the number of mammals used for projects on '3Rs and research' have remained stable. However, the number of animals used for regulatory compliance fluctuates from year to year. This is because of changing regulatory demands, which can be impacted by global regulations coming into force.

Figure 1 Purpose of testing in mammalian species

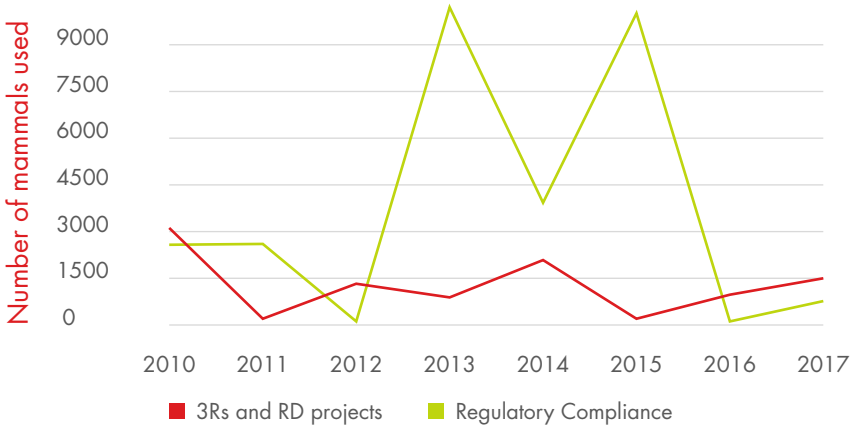


Figure 2
2013 – 2017 Tests for regulatory compliance

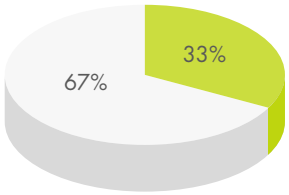
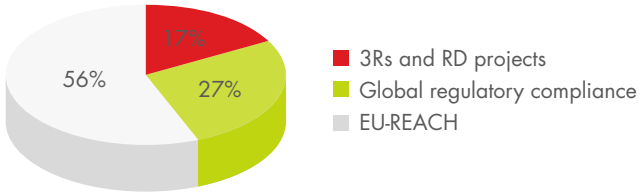


Figure 3
Purpose of Testing
2013 – 2017



In 2017, the total number of mammals in tests were done under industry consortia (see Table 1). From the 1,790 rodents and rabbits used 33% was to comply with the EU chemicals legislation REACH. As seen in Figure 2, in the last five years approximately two thirds of all regulatory mandated tests were done to comply with the EU chemicals legislation.

Testing is also performed to advance the 3Rs or for research purposes (Table 3). As of 2017, the term '3Rs and research', replaced the term 'product stewardship' used in previous reports. As indicated previously '3Rs and research' refers to data generated to understand the health and environmental hazards of a product which is not mandated for regulatory compliance. This data is also used or generated to advance 3R methods and may include generation of detailed information on the mechanism of toxic action that is informative about the relevance of the used animal model for human risk assessment. For the period 2013-17, 17% of experimental animals reported by Shell were for used for research and 3Rs projects. Details in these research projects is reflected in this report under 'research and development'.

TESTING IN FISH SPECIES

3R and research1 activities in ecotoxicology testing include specific studies on mode of action and species sensitivity distributions, which help to reduce the number of standard tests needed under mandatory regulatory requirements. In 2017, 274 fish and 17 amphibians were used for 3R and research purposes. In addition to product safety testing, some countries (particularly the USA and Canada) require the use of fish to assess the toxicity of discharges into water. Operating permits for industrial sites, such as oil refineries, chemical plants, supply and distribution terminals, and retail sites require the toxicity of effluent waters to be tested in a range of aquatic organisms, including fish. In 2017, this amounted to over 99% of all fish tested. This continues to be the largest driver of animal use numbers for across Shell for all vertebrates (mammals, amphibians and fish) at 94%. Table 3 presents a five-year overview of the numbers of fish required to comply with regulatory requirements and those used for 3R and research purposes. Vertebrate numbers for both 3R and research, and regulatory compliance decreased significantly in 2017 because Shell divested its oil sands operations as of June 1, 2017.

Table 3 Use of fish, 2013-2017

Purpose of test	2013	2014	2015	2016	2017
3Rs and research[1]	11,326	25,960	18,589	8,480	274
Regulatory compliance	48,966	56,533	66,867	46,871	34,378
TOTAL FISH	**60,292**	**82,493**	**85,456**	**55,351**	**34,652**

[1]3Rs and research: data is required to understand the health and environmental hazards of a product and is not collected for direct regulatory purposes. This may include generation of detailed information on the mechanism of toxic action. This mechanism of action can inform the relevance of the used animal model for human and environmental risk assessment. This testing is also performed to help Shell understand the potential implications of anticipated future regulatory requirements or applications for new permits (discharges).

Fish use in the water return project 2013-2017

Over the last five years, Shell has tested on a large number of vertebrates in its oil sands operations through its water return programme. The total number of 44,234 fish was included under 3R's and research in Table 3, spread over the 2013 – 2107 period. The goal of this programme was to build a case for the safe return of process water to the environment by achieving a net benefit to the surrounding environment, instead of maintaining these waters on-site in tailings ponds. Although Shell's oil sands operations were a JV, as a Shell-driven initiative the vertebrate numbers were reported as "Shell only" since the start of the project in 2013. However, in the 2016 report, 4,800 fish from the water return programme were mistakenly reported as coming from this JV. Although this is formally true, to maintain consistency with earlier reporting, we are amending the 2016 numbers so that these 4,800 fish are reported as "Shell" in the 2016 report. As this is a reassignment of numbers resulting in higher fish numbers for Shell and less animals for the JVs, the overall number of vertebrates in 2016 remains the same. Additionally, it must be pointed out that within the Shell water return project initiative agreement for data sharing, all JV partners, along with all other oil sands industry operator peers under the Canadian Oil Sands Industry Alliance (COSIA), will have access to the generated data.

Zebrafish

All data has been presented at scientific conferences and workshops and/or published in the peer-reviewed scientific literature (e.g. Galus et al 2017, Huang et al., 2018; Hughes et al., 2017b; 2017d; Kilgour et al., 2017) with additional work being published into 2018. Data was also presented internally to all oil sands operators through COSIA at numerous internal meetings related to oil sands process water. Finally, all data was provided to the company who purchased Shell's oil sands operations in 2017.

ABREVIATIONS

3Rs Replacement, reduction and refinement of tests that use animals
CEFIC European Chemical Industry Council
CONCAWE The org anisation of environmental science for the European refining industry
DART Developmental and reproductive toxicity
ECETOC European Centre for Ecotoxicology and Toxicology of Chemicals
EU European Union
FT Fischer-Tropsch synthesis process
GC Gas chromatography analytical technique
GTL Gas-to-liquid substances produced by FT
HESI Health and Environmental Sciences Institute
ILSI International Life Sciences Institute
NC3R UK National Centre for the replacement, refinement and reduction of animals in research
OECD Organisation for Economic Co-operation and Development
PAC Polycyclic aromatic compounds
PBT Persistent, bioaccumulative and toxic
PETROTOX A model that predicts the aquatic toxicity of complex petroleum substances from petroleum substance composition
QSAR Quantitative structure activity relationships model
REACH The European Union regulation No. 1907/2006 concerning the registration evaluation, authorisation and restriction of chemicals
SPME-GC Solid phase micro extraction with gas chromatographic analysis
TSCA The Toxic Substances Control Act of the United States of America
UVCB Substances of unknown or variable composition, complex reaction products and biological materials
WAF Water accommodated fraction methodology

ABOUT THE PANEL

In 2001, Shell formalised its practices on animal testing by creating a more structured management process and by better communicating its position internally and externally. An external Animal Welfare Panel was established to provide independent scrutiny of, and support for, Shell's activities in this area.

TERMS OF REFERENCE OF THE PANEL

Individual panel members are invited by Shell to serve on the panel for a period of three years, with the possibility of being invited to serve for a second term of three more years. The panel recommends candidates who could be invited by Shell to join the panel, either as replacements for current members when their term has been completed, or to supplement the current panel membership.

The panel meets twice a year with key Shell personnel. It does not verify the accuracy of the data underlying the report. Besides assessing Shell's reporting on animal testing, the panel offers observations and advice on the company's performance with respect to the 3Rs. In recognition of their time and expertise, panel members receive an honorarium and reimbursement of travel and accommodation expenses.

PANEL MEMBERSHIP IN 2017

Charles Gentry (independent consultant on laboratory animal science), Panel Chair
Charles Gentry is a company director with international expertise in laboratory animal science. He has a specialist interest in compliance with UK and EU legislation, and in the implementation of good practice. He is a former Director and Certificate Holder under A(SP) A 1986 at the University of Cambridge, UK. He is Chairman of the Establishment Licence Holders Committee UK, Chairman of the Animal Health Trust Animal Welfare and Ethical Review Committee UK, compliance consultant to the British Antarctic Survey, and a member of the Home Office Advisory Group on Laboratory Animal Science.

Catherine Willett (Director, Science Policy, the Humane Society of the United States)
Kate Willett began her career at the Massachusetts Institute of Technology as a developmental biologist studying embryology using the zebrafish as a model system. She then joined a start-up company that pioneered the use of zebrafish for preclinical drug testing. Since 2006, she has focused on the science, policy and regulatory aspects of replacing animals as the basis of chemical safety assessment, first as Science Policy Advisor for People for the Ethical Treatment of Animals, and more recently at the Humane Society of the United States as coordinator of the Human Toxicology Project Consortium (HumanToxicologyProject.org). She has published numerous papers on non-animal approaches and advises international companies and governments on the regulatory use of non-animal methods.

Jim Bridges (Emeritus Professor of Toxicology and Environmental Health at the University of Surrey, UK)
Jim Bridges held previous positions in the University of Surrey, including Dean of Science and founding head of two large health research and teaching institutes. He has published nearly 400 papers and reviewed and trained 98 PhD students. He is a founder of both the British Toxicology Society and EUROTOX. His work for the EU included as Chair of two scientific committees – Emerging and Newly Identified Health Risks; and Toxicity, Ecotoxicity and the Environment – as well as several working groups on future risk assessment methodology that have addressed alternatives to animal testing.

Robert Hubrecht (Chief Executive and Scientific Director – Universities Federation for Animal Welfare & the Humane Slaughter Association)
Robert Hubrecht is an ethologist with an interest in animal welfare. Prior to joining the Universities Federation for Animal Welfare, he held positions at the Open University and Cambridge University in the UK. His research has included studies of the behaviour, physiology and natural history of farm animals, New World primates (both in captivity and in the wild), and the welfare of kennelled dogs. He has served on numerous advisory committees, including the UK Animal Procedures Committee, the US National Research Council Distress Committee, and expert groups that provided advice on the development of UK and European legislation. He co-edited the 8th edition of The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals. In 2014, he authored the book: The Welfare of Animals Used in Research: Practice and Ethics

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