Shell External Animal Welfare Panel report 2015

We have made a one-off amendment to the date of this year's report to bring it in line with other Shell Group annual reports. The Animal Welfare Report for 2015 contains details and data for activity carried out during 2015. Note that this contrasts with previous years where the title year referred to the year of report publication.

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EXECUTIVE SUMMARY

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

Shell continued the development of innovative non-animal test methods for assessing product safety, such as alternative screens for developmental and reproductive toxicity. By sharing our experiences with innovative methods at conferences and by publications in peer-reviewed journals, we are contributing to the growing momentum for global regulatory acceptance of these 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.

Regulatory compliance remains the main reason for animal testing, especially in chemical safety testing for the EU regulation on Registration, Evaluation, Authorisation and restriction of Chemicals (REACH), and effluent testing in the US and Canada.

Shell has achieved substantial reduction of animal testing, especially fish, by using read-across and grouping strategies. There are further opportunities to reduce the number of mammals used in safety testing - particularly in the area of developmental and reproductive toxicity assessment.

In 2015 the total number of animals used was 101,229. Of this total, 10,003 were mammals, 85,456 were fish, and 5,770 were amphibians.

Shell's priorities in 2016 will be to:

- Develop innovative tools to assess reproductive and developmental toxicity.
- Develop test assays that help reduce the environmental impact of oil sands operations and at the same time reduce the number of fish in effluent testing.
- Continue engaging in consortia at industry level as well as with regulators in order to advocate the use of alternative animal testing strategies.
- Participate in scientific fora, publish our research findings and educate the student community and the public about the advances, limitations and opportunities in improving animal welfare in the context of chemical safety assessment.

INTRODUCTION

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

"Congratulations on your efforts in delivering the report to this excellent standard and a perfect example of a proactive way of communicating your animal research and engaging more thoroughly with the public and your own staff"

The Animal Welfare Panel

Shell implements the 3Rs of animal testing (replace, reduce, refine) wherever possible while meeting legal obligations and protecting human life as well as the environment. Any Shell-owned or Shell-operated company must follow the company's animal testing standards when performing laboratory-based toxicology experiments on animals, even in countries that have less stringent requirements.

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 best practice in laboratories. It also advises on how Shell should engage 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 document 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 alternative non-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.

DYNAMIC OF ANIMAL WELFARE ACTIVITIES IN SHELL

Regulatory compliance remains the main driver for animal use in Shell. The approach to animal welfare can be grouped into four activity circles. Each 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.

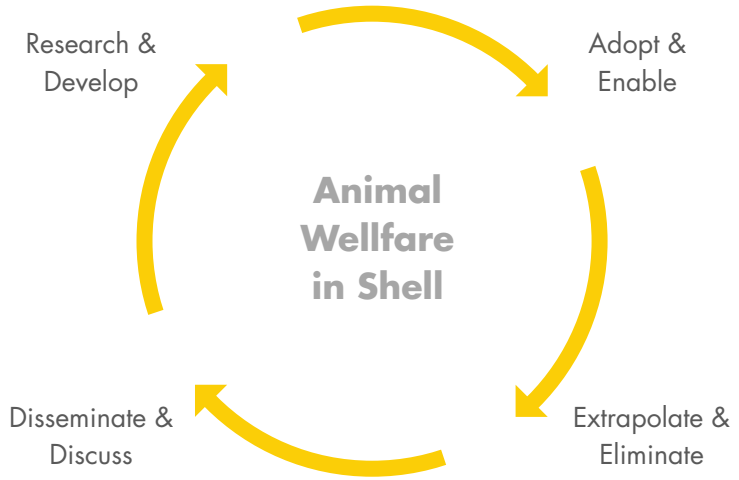
Research and Develop are efforts related to collaboration, funding and conducting research for innovative hazard and exposure assessment methods. Drivers for prioritisation are business need, and areas where the highest impact on the 3Rs can be achieved.

Adopt and Enable aims at applying our Research & Develop advances, learnings and best practices by others into Shell's practice. 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 minimising animal use by leveraging of data. Integration of information from multiple sources can be achieved by establishing, utilising and maintaining access to databases. Collaboration with external parties for this is essential.

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

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



RESEARCH AND DEVELOP

Our research priorities in 2015 were driven by business needs and areas where Shell could impact most on animal use.

The core nature of the business – oil and gas – means most substances produced by Shell are derived from crude oil and are therefore known as UVCBs: substances of *Unknown or Variable composition, Complex reaction products and Biological materials.* These substances are manufactured against physico-chemical specifications (for example boiling point range) rather than a specific chemical composition.

Globally, there are approximately 600 to 700 individual petroleum substances. Although UVCBs vary in chemical composition, they can be grouped into categories as their refining history will drive the presence of specific groups of constituents with known hazard profiles. Petroleum substances in one category can be considered to have a comparable chemical composition and hence a comparable hazard profile. As chemical regulations are designed for single substances, the categorisation and hazard assessment of petroleum substances under these regulations remain a challenge.

Research on UVCBs in specific petroleum substances and grouping, categorisation, and read-across strategies for these substances are therefore priorities for Shell.

Assessing the tests Shell performs involving animals, as well as emerging chemical safety regulations, it is clear that most mammals are used for reproductive toxicity testing. It is also clear that most fish are used for mandatory effluent testing in the North-America region. This drives research and development of alternative methods in these areas as a priority.

Development of Non-Animal Methods for Human Health Protection

Grouping and read-across strategies

Research on petroleum substances, in specific their grouping, categorisation, and read-across strategies for these substances can drive significant progress in the 3Rs. If solid strategies can be developed for both categorisation and read-across, this will avoid the testing of all 600 to 700 individual petroleum testing and thus save a significant number of animals. In the current grouping approach, existing toxicological data of some UVCB substances is used to read-across to other category members. This approach may be challenging because the applicability domain of the data requires good understanding of the variability of constituents in a UVCB substance within a category. In addition, many gaps in the available toxicity data preclude confident groupings of these substances for read across applications.

A grouping strategy applied by a REACH consortium that Shell participates in, is comprised of a strategy for 32 UVCB petroleum-derived substances. These substances consist of similar constituents but have different carbon chain lengths (e.g. C6-C40). The regulator requested data for these substances on the prenatal development and repeated dose endpoint. A developmental toxicity test following OECD 414 will typically use around 1,300 rats (including offspring). A repeated dose toxicity test following OECD 408 will typically use 80 rats.

In the worst case, testing 32 individual UVCB petroleum-derived substances would use a total of approximately 44,160 rats for these tests alone. The consortium has applied a testing strategy, in which

testing would cover the low, middle and high end of a substance category in regards to its carbon chain lengths. By application of substance grouping and this testing strategy, five prenatal development studies and five repeated dose studies were performed to reliably demonstrate product safety. This saved prenatal development and repeated dose studies for 27 individual substances and a total of 37,260 animals.

Supplementing a grouping and read-across strategy with non-animal test data can also contribute to the reduction of the number of animals. It can also strengthen the read-across strategy.

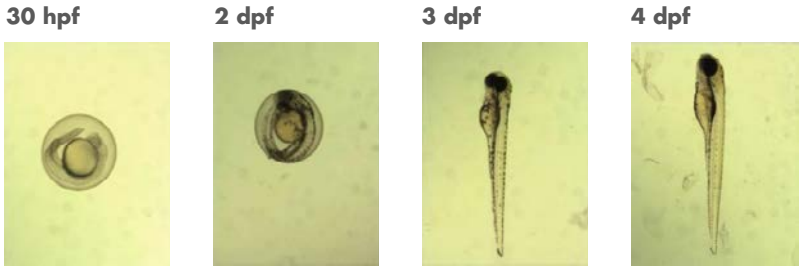
Shell is currently developing a comprehensive experimental and computational approach to categorising petroleum substances as model UVCBs according to global similarities in both their chemical composition and their bioactivities using a suite of *in vitro* models (Boogaard, 2015).

To support read across by chemical similarity, the substance-specific composition of a set of petroleum substances was determined using novel analytical chemistry techniques. The outcome of the substance analysis could confirm placement of the petroleum substances in categories of similar chemical composition. Representative petroleum substances from each category were tested *in vitro* for biological read-across. The observed effect served as a biological tool to evaluate similarities and differences both within and across different substance categories.

Results of the experiments allowed definition of substance categories based on a high degree of correlation between biological and chemical data sets. Altogether, it was demonstrated how novel analytical chemistry and *in vitro* screening approaches can be effectively combined to categorize UVCBs thereby indicating their potential applicability in regulatory submissions and potentially reducing the number of traditional in vivo regulatory tests (Grimm et al., 2015 and 2016).

Developmental and reproductive toxicity

As testing for reproductive and developmental toxicity could potentially require significant use of animals, this has been a 3Rs research focus for Shell. In a recent publication (Tsitou et al., 2015), Shell proposed the adoption of a battery of *in vitro* tests for the evaluation of reproductive toxicity of petroleum UVCBs that utilises the zebrafish embryo test, embryonic stem cells and the whole embryo culture.



Zebra fish embryos at 30 hours post fertilization, and 2,3 and 4 days post fertilization.

Zebra fish

The amoeba Dictyostelium discoideum (left) and the nematode Caenorhabditis elegans (right), are used as model organism for the assessment of developmental and reproductive toxicity.

Beyond the applicability of the zebra fish embryo test for ecotoxicity testing of petroleum substances, this model is also under investigation for its application in assessing potential human health hazards of these substances (Muriana et al., 2014).

In addition, the zebrafish embryo test can be used for the assessment of DART (developmental and reproductive toxicity) with invertebrate model organisms such as nematodes (*C. elegans*) and amoeba (*D. discoideum*) in a combinatorial testing strategy.

Although these organisms are different species and may show different sensitivity to DART compounds, the basic assumption is that key adverse outcome pathways (AOP) for reproductive and developmental effects have been conserved across species, including humans. Alterations of these AOP are measured as indicative of the potential DART properties of the tested compounds in humans (Steup et al., 2015).
Shell is co-sponsor of the UK NC3Rs CRACK-IT project 'PREDART', investigating this test battery (Smulders, 2015). The project is expected to be completed in 2016.

Development of Non-Animal Methods for Environmental Protection

Biodiversity assessments are a common feature of offshore oil and gas industry environmental monitoring programs.They are mainly used to assess potential adverse impacts of drilling operations. Shell is assessing the potential for environmental DNA (*eDNA*) methods to provide information about the ecological community at the seabed level from deep-water sediments. Approaches like DNA barcoding and *eDNA* could potentially be less expensive, safer, and more thorough than the traditional methods in which organisms are isolated from sediment samples by sieving, preserved and subsequently identified by experts. Preliminary results show that the technology could detect the presence of *eDNA* from a diversity of marine species, as well as from terrestrial and freshwater species, which had probably been deposited by river outflow. A high percentage of DNA could not be identified, but it was determined to be mainly from marine organisms. The technology is subject for further investigation (Bickham et al., 2015; Stoeckel et al., 2015).

ADOPT AND ENABLE

For the adopt and enable activities, Shell has focused on the application of existing non-animal methods, and enabling their use for Shell-specific substances, like petroleum substances. Most currently accepted non-animal assays heavily rely on aqueous solutions, whereas petroleum substances are poorly-water soluble. In addition, existing computational models and read-across strategies tends to focus on single chemicals. Petroleum substances are however substances of *Unknown or Variable composition, Complex reaction products and Biological materials* (UVCBs), hence some modifications could be needed for effective application of computational models or read-across strategies.

Application of Non-Animal Methods and Testing Strategies for Human Health Protection

***In vitro* skin and eye irritation and skin sensitisation**

In the European chemicals legislation REACH, the assessment of skin corrosion or irritation, is a standard requirement beginning from lowest tonnage band (more than one tonne a year). In vitro methods including the reconstructed human epidermis test method for skin corrosion (OECD 431) and for skin irritation (OECD 439) are the first choice to fulfill the information requirements.

For the REACH registration for a multi-constituent petrochemical substance, eye and skin corrosion or irritation were part of the standard information requirements. For the assessment of skin corrosion and irritation a tiered approach was followed using the OECD 431 and OECD 439 guidelines respectively. Eye irritation and corrosion was assessed using the Bovine Corneal Opacity and Permeability test method for identifying ocular corrosives and severe irritants.

The substance was not corrosive in the *in vitro* skin corrosion test, but it was irritating to the skin fulfilling the criteria for classification and labelling. On the other hand, results from the *in vitro* eye corrosion test were negative, but did not allow concluding on whether the substance might fulfil the classification and labelling criteria for mild eye irritation. Therefore, an in vivo eye irritation test using the minimum number of rabbits (OECD 405) was conducted. It established that the substance was not a mild eye irritant and did not require classification and labelling.

It is concluded that for the evaluation of this multi-constituent petrochemical substance the available *in vitro* skin corrosion or irritation tests perform well allowing conclusive classification and labelling. Further investigations on the applicability of the *in vitro* skin assays for assessment of petroleum substances will take place in 2016.

For the assessment of eye irritation effects the available *in vitro* eye irritation tests have their limitations when assessing potential mild irritants. Currently available *in vitro* eye irritation tests, additional to the Bovine Corneal Opacity and Permeability test method, remain an opportunity for further investigation for petroleum-derived substances.

In vitro methods including the reconstructed human epidermis test method are the first choice to fulfill information requirements.

In 2015, ECHA has published advice on using new OECD test guidelines related to skin sensitisation. These new non-animal test guidelines each address a specific key event in the adverse outcome pathway for skin sensitisation, and can replace the need to use animal test methods. The adopted OECD test guidelines are the Direct Peptide Reactivity Assay (DPRA, OECD 442C), KeratinoSens™ (OECD 442D), and the In Vitro Human Cell Line Activation Test (h-CLAT, OECD 442E).

The new tests, however, rely on aqueous solutions for substance exposure and as such may pose a challenge when assessing poorly water soluble petroleum substances.

Results from testing petroleum substances in the DPRA are inconclusive due to precipitation or inaccurate dosing as a result of poor solubility. As the DPRA gains acceptance as a validated alternative method, such technical difficulties in its applicability domain need to be acknowledged and solutions found.

Considering this technical challenge, new petroleum-like substances are still tested in guinea pig tests (Reitman et al., 2015) because their assessment *in vitro* has proven technically not feasible. Similar solubility issues may also be encountered in a number of other alternative methods that use aqueous solutions.

The modification of these tests to expand their applicability domain for petroleum UVCB substances has been identified as an opportunity for Shell to implement the 3Rs. Shell has engaged in a research project to modify the current protocols for the DPRA to accommodate poorly water soluble petroleum substances. Preliminary results are expected by the end of 2016.

The use of QSAR modelling has been successfully implemented in the registration of lubricant base oils for offshore applications in Norway

Application of Non-Animal Methods and Testing Strategies For Environmental Protection

Quantitative Structure-Activity Relationship Models (QSAR)

The use of QSAR modelling has been successfully implemented in the registration of lubricant base oils for offshore applications in Norway.

In order to register the base oil components of a range of Shell hydraulic fluids for use in oil and gas field operations offshore Norway, a full suite of marine species toxicity data (including fish) is required.

Highly-refined lubricating base oils are known to be non-toxic to aquatic organisms because of their extremely poor water solubility (lack of bioavailability). Therefore, to satisfy the specific Norwegian regulatory requirements for the specific base oils in the Shell formulated hydraulic fluids, it was decided to only generate experimental data for an invertebrate sediment dwelling species. For the aquatic species toxicity data requirements a read-across and QSAR (CONCAWE PETROTOX model, https://www.concawe.eu/reach/petrotox) approach was adopted, along with scientific arguments to waive the fish toxicity testing requirements.

This alternative approach to experimental fish testing (QSAR and read-across) is already extensively used under EU REACH and some other regulatory notification schemes (for example, the US Environmental Protection Agency Toxic Substances Control Act notification process). QSAR models like PETROTOX for predicting acute and chronic aquatic toxicity of chemicals with a well-defined mode of action are well validated and have gained regulatory acceptance. Its use under the Norwegian regulations provided Shell with the opportunity to promote better use of alternative read-across and QSAR approaches.

The alternative read-across and QSAR strategy is estimated to have saved approximately 70 -250 fish.

The approach taken by Shell to waive the requirement for experimental fish toxicity testing was accepted by the Norwegian Environment Agency. This is clearly a successful demonstration of the Replacement of animals in line with 3Rs principles.

GTL solvents have been registered in the USA using a read-across strategy and new analytical data.

Read across

For registration of a series of eight Gas-to-Liquid (GTL) solvents in the United States, Shell followed a read-across strategy by using existing ecotoxicity data on related GTL products that overlapped the carbon ranges of the solvents intended for registration. In the screening models used by the US Environmental Protection Agency (EPA) some constituents in the GTL solvents are estimated to have a high water solubility and are therefore assumed to be a potential driver for chronic toxicity even at very low concentrations. Therefore, additionally to the read-across strategy new analytical data of all solvents was provided, measured at the predicted low concentrations of suspected toxicity. The read-across was supported by testing of the two most soluble solvents as representation of "worst case" in chronic fish, daphnia, and algal toxicity tests. In the end, no chronic toxicity was seen in the tested samples and the data from these two solvents were used as read-across strategy to the other solvents, saving 2880 fish (Hughes et al., 2015). The US EPA accepted this data and all eight GTL solvents were allowed to be registered.

EXTRAPOLATE AND ELIMINATE

The extrapolate and eliminate activities focus on minimising the use of tests involving animals by the leveraging of data, for example through replication of learnings and successes across regulatory frameworks. Collaboration with external parties for this is essential.

Minimizing animal use by leveraging of data

A REACH Consortium conducted a required study in rats on a metal used as a catalyst to evaluate reproductive and developmental toxicity for REACH registration. The Shell toxicologist providing oversight for this study, expressed concern over the fact that, according to the protocol, groups of 25 rats were treated to ensure 20 pregnant females per group were available for evaluation at the end of the study. The excess of 20 females (five from each group of 25) and any pups would then be sacrificed and discarded. Working with the sponsoring group and the contract laboratory, arrangements were made to analyse these "extra" animals for additional control data or to assign them to other studies where they could generate useful information. The additional rats ultimately could not be spared, but were utilised productively to improve results and possibly spare others in the future.

An example of minimising animal use, includes a study using animals that were bred for food consumption.
Shell has investigated the use of certain grass species for the production of bioethanol (to serve as biofuel). To ensure sustainability of the technology, Shell investigated whether the resulting silage is useful as animal feedstock. The feeding study was conducted at a university facility in the USA, and included 106 beef steer specifically bred for meat production. By using animals that were already bred for meat production, Shell avoided unnecessary use of laboratory animals. The study took place under the supervision of the University Institutional Animal Care and Use Committee (IACUC). It has given Shell valuable insight on how to maximize the use of biomaterial, not only for biofuel production, but also by returning the silage back into the food chain.

Replication of learnings and successes

The success story of the registration of GTL solvents in the USA was successfully replicated in the EU where several GTL solvents were registered under REACH in 2015.
At the volumes registered both acute and chronic fish toxicity data were required. Using a category and read across approach for these products, these registrations were completed with no additional testing performed, thereby preventing six acute fish tests and seven chronic fish tests and thus a total of 3,612 fish.
As the REACH process matures, examples like this one strengthen the case for read across and category approaches, accepted by REACH. It will however, still require subject matter expertise and regulatory scrutiny. Shell will pursue this approach leveraging its expertise and experience when working in consortia (Lyon et al., 2015).
- Collaboration with external parties - work through consortia
The European Chemicals Regulation REACH requires companies to share data and hence avoid unnecessary animal testing. The practical implementation by the European Chemicals Agency of the data sharing requirement is the creation of Substance Information Exchange Fora. In addition, industry has formed consortia dealing

with specific substances, in which the research and potential animal testing is identified and discussed by subject matter experts in (eco)toxicology and animal welfare. This enables inclusion of 3Rs principles in all testing proposals. The sharing of the data prevents that animal tests have to be replicated by each individual member of the consortium or Substance Information Exchange Forum. Shell will work through consortia where possible, even if this is not mandated by law.

DISSEMINATE AND DISCUSS

Shell publishes animal numbers, results from (non)animal testing, and new approaches developed either independently or within a consortium with the aim of transparency and to share data and best 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 instill a culture of care at the highest scientific and practical levels.

Shell's external engagement in alternative animal testing methods

CHINA

In China, new chemical safety regulations are under development. This provides opportunities to share learnings and experiences from chemical safety regulations from other regions.

In July 2015, Shell, together with Unilever, L'Oréal and Humane Society International, co-organised the first *"International Conference on Toxicological Alternatives & Translational Toxicology"* in Xian, China.

The conference was hosted by the Chinese Society of Toxicology's Committee on Toxicological Alternatives and Translational Toxicology (CSOT) and the Chinese Environment Mutagen Society's Committee on Toxicity Testing and Alternative Methods (CEMS).

More than 500 experts and scientists from industry, research institutes as well as government around the world attended. The conference served as international platform for knowledge exchanges and in-depth debates between scientists, industry and government representatives.

Shell chaired a special China Roadmap session: "Toxicity Testing in the 21st Century - TT21C" and the use of "Adverse Outcome Pathways – AOP". It aimed to share experiences and increase China's regulatory uptake of currently available alternatives, including AOP (Chemical Watch, 2015; Niven, 2015; Willett, 2015). This special session attracted a balanced representation of interest to discuss non-animal testing approaches in toxicity testing, and established a TT21C/AOP working team to focus on developing a China AOP roadmap.

CANADA

In October 2015 two Shell ecotoxicologists chaired a session on alternatives to vertebrate testing at the 42nd annual Canadian Ecotoxicity Workshop in Saskatoon, Canada. The session was titled *"Expanding Horizons – Alternative non-vertebrate test methods for evaluating ecotoxicity"*, the aim was initiate discussion and get the latest thinking on this issue. The session was fully booked with a mixture of academic, industry and government representatives. Shell presented its strategy to reduce vertebrate testing and shared best practices.

Canadian policy still relies on whole-effluent toxicity testing using fish for ensuring the safety of effluent discharges, similar to the USA. Environment and Climate Change Canada, the Canadian government's environmental agency, shared that it takes about a decade for an alternative test method to be developed, tested and approved. It also shared results of trials with screening tests *(e.g. microtox)*, which indicated that these tests do not consistently replicate whole organism tests' responses. They recommend developing new alternative methods and suggest appropriate ring testing of these. Academia and contract laboratories were challenged to consider these novel methods and possible commercialisation of novel non-vertebrate methods. Shell encouraged early engagement and discussion with regulatory agencies, such as Environment Canada, for the potential adoption of new test methods. This workshop session created awareness and support for the 3Rs in animal testing with the research and regulatory community in Canada.

The outcome of this session was to host a second session on animal alternatives for the 43rd Canadian Ecotoxicity Workshop meeting in 2016 to build upon the momentum of the 2015 session. Additionally the 2015 session led to an invitation to be involved in the successful Genome Canada grant application with a multi-university research team for development of an *EcoToxChip*: a toxicogenomics tool for chemical prioritisation and environmental management. This genomics tool is aiming to replace animal testing for chemical screening and effluent testing.

Rick Scroggins (Chief of the biological methods section for Environment and Climate Change Canada) approached one of the Shell ecotoxicologists and remarked:

"An excellent session and about time that we had this discussion in Canada."

Shell Use of Animals for Testing in 2015

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 participates are reported separately. Shell reports all experimental animal use on a 100%-basis (each animal is counted as Shell's 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, 2011 – 2015

Animal used	Tests commissioned	Number of animals per year				
		2011	2012	2013	2014	2015
Fish	Shell	33,753	30,832	44,696	61,773	76,476
Fish	Industry consortia	0	4,368	5,576	0	2,720
Fish	Joint ventures	11,763	4,180	10,020	20,720	6,260
Amphibians	Shell	0	0	0	0	5,770
Rodents	Shell	2,497	150	4,368	2,591	72
Rodents	Industry consortia	748	7,944	5,763	3,202	9,908
Rodents	Joint ventures	0	0	0	0	0
Rabbits	Shell	6	9	870	40	3
Rabbits	Industry consortia	0	6	4	0	20
Rabbits	Joint ventures	0	0	0	0	0
Birds	Shell	90	0	0	0	0
	TOTALS	**48,857**	**47,489**	**71,297**	**88,326**	**101,229**

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 joint ventures where Shell has operational control.

A three-year research program will use wood frogs to study the impact of oil sands process-affected water on amphibians.

The total number of laboratory animals used between years 2011-2015 is shown in Table 1. For 2015 the total number is 101,229.

In 2015 the use of fish for regulatory mandated effluent testing in North-America remains the most significant contributor to the total number of animals used by Shell.

Amphibians have been used for environmental studies as part of a three-year research program. This program investigates the impact of oil sands process-affected water on amphibians. Due to their complex life cycle there is concern that amphibians might be more sensitive to contaminants than other species. In addition, as wet-landscape approaches are under consideration for post-mine reclamation, frogs will be an integral species in the ecosystem.
In the research program novel techniques will be implemented in addition to traditional toxicity testing to help develop alternative testing methods for understanding exposure and effects in amphibians.

The use of mammals in tests by Shell has significantly decreased when compared to increasing numbers used in industry consortia.

The benefit of performing animal testing through consortia is that data are shared between the consortium participants. This enables agreement of study design upfront and prevents comparable animal tests to be performed by each individual consortium participant. The specific impact on animal testing numbers is shown in Table 2. In 2015, a total of 10,003 mammals were used for testing, of which 9,928 for testing in consortia. Shell reports on a 100% basis, meaning that all animals used in a specific consortium are counted. However, if the number of animals used in a consortium study would be divided by the total number of consortium partners, this would reflect the actual 'Shell share' of the number of animals used. This is reflected in the last column of Table 2. The calculation shows that of a total of 9,928 mammals used in consortia, the 'Shell share' is 1113 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	9,940	9,888	1111
Mice	40	20	1
Rabbits	23	20	1
TOTAL	**10,003**	**9,928**	**1113**

PURPOSE OF TESTING ON ANIMALS IN 2015

The purpose of performing tests on animals is summarised in Table 3. The main driver for testing is regulatory compliance. REACH was the only regulatory framework for mammalian testing in 2015, whereas effluent testing remains the main driver for tests involving fish. Product Stewardship activities on animal testing include specific studies on mode of action, which help reduce the number of standard tests needed under standard regulatory requirements (see section on "Regulatory Acceptance and Use of Non-Animal Methods and Testing Strategies".)

Table 3: Mammalian use by purpose

Test Purpose	Number of mammals
Product Stewardship*	118
Regulatory Compliance	9,885
TOTAL	**10,003**

* **Product stewardship:** Data is required to understand the health and environmental hazards of a product and is 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 shown in Figure 1, the number of mammals in tests used for regulatory compliance has significantly increased since 2010 when the EU chemical regulation REACH came into force. Animal testing for REACH compliance is done primarily through Industry Consortia.

Total numbers of animals used vary from year to year, but there is an upward trend that is expected to continue as ECHA will require testing on reproductive and developmental toxicity. Shell will continue to propose and use alternative testing strategies to reduce the number of animals required for product safety where possible within the regulatory framework.

Figure 1: Purpose of Testing in Mammalian Species

Explanatory Notes:

Product stewardship: Data is required to understand the health and environmental hazards of a product and is 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.

Regulatory compliance: Testing is required by law.

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.

Table 4 presents a five year overview of the numbers of fish required to comply with regulatory requirements and those used for product stewardship purposes. The number of fish required for regulatory permits comprises 73% of the total number of fish used in 2015.

Fish testing for Shell and for product stewardship has increased since 2013. The main reason has been a three-year project, nearing completion, for the oil sands operations. This aims to reduce effluent testing in the longer term and to enable water to be returned to the environment safely.
Oil sands operations are required to contain all site process-affected waters in external tailings facilities (ie pools where contaminated waters are contained). This includes all process waters and groundwater streams that are not used in processing but are in contact with the bitumen ore when mining. The water return project has been a multi-year study to characterize 10 different water streams on site with full water chemistry and a suite of Environment and Climate Change Canada effluent tests that require fish. Developing water stream profiles allows to predict toxicity enabling the return water that has been tested to be safe to the environment. In this way a normal hydrological cycle is maintained with the river from which freshwater is taken for ore processing. This project will help us to reduce fish numbers in effluent testing at the mines and will lower the overall environmental footprint of the oil sands operations.

Other contributors to increased fish use in 2015 are the completion of environmental and effluent testing programmes for off-shore projects in the Gulf of Mexico and Africa.
The project in the Gulf of Mexico is a deep-water well under development, which because of the extreme operating conditions will use complex combinations of production chemicals. The effluent discharge containing these chemicals has to be tested for safe discharge as a part of the discharge permit requirements.
The project off the coast of Africa has the regulatory requirement for endemic fish species to be used for effluent testing. This requirement caused increased fish use and limited the use of alternative approaches.

Table 4: Use of fish and amphibians, 2011-2015

Purpose of test	2011	2012	2013	2014	2015
Product stewardship	17	5,060	11,326	25,960	18,589
Regulatory compliance	45,029	34,320	48,966	56,533	66,867
TOTAL	**45,516**	**39,380**	**60,292**	**82,493**	**85,456**

CONCLUSION

The Shell External Animal Welfare Panel endorses the achievements of Shell in:

- Identifying key activities which aim to improve animal welfare and only use animals where there is currently no practical alternative
- Reducing animal use significantly by using read-across and chemical grouping, and assessing toxico-genomic responses and the use of *in vitro* tests. Working through consortia and industry groups allows Shell to promote the culture of care and the 3R mindset
- Engagingwith other relevant external organisations and publish its research thereby raising awareness and acceptance of alternative methods in chemical and environmental safety assessments.

The Panel notes that:

- Regulatory compliance remains the key driver to conduct animal testing
- Although not mandated by law, internal product stewardship projects allow advancing the science, validate data and apply best practice. The controlled used of animals in these projects will allow significant reduction of tests in the future.

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NOTE: Poster and presentations are available upon request.

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 2015-16

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. Mr Gentry 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, Regulatory Toxicology, Risk Assessment and Alternatives, 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 and 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 HSUS and as coordinator of the Human Toxicology Project Consortium (HumanToxicologyProject.org). She has numerous publications on non-animal approaches and advises international companies and governments on the regulatory use of non-animals methods.

Jim Bridges (Emeritus Professor of Toxicology and Environmental Health at the University of Surrey, UK)
Jim Bridges held previous positions in 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 reviews and trained 98 PhD students. He is a founder of both the British Toxicology Society and EUROTOX. Work for the EU included the Chair of the 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 interests in animal welfare. He has held positions at the Open University and Cambridge University prior to joining the Universities Federation for Animal Welfare. His research has included studies of the behavior, 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, and in 2014 authored the book: The Welfare of Animals Used in Research: Practice and Ethics, Wiley Blackwell.

PHOTO CREDITS

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