Report on Non-Animal Tests for Low Risk Psychoactive Substances

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This report is based on a submission to the health select committee by the New Zealand Anti-Vivisection Society (NZAVS) and was prepared at the request of the Huha Animal Sanctuary. It includes detailed information on validated non-animal tests suitable for the safety testing of psychoactive drugs and outlines the problems with relying on animal testing data. As an appendix it has a report prepared by two regulatory and toxicology testing experts commenting on advice the Ministry of Health requested that proposed a testing regime based on the use of animals. This report concludes that the introduction of a ban on the use of animal testing data would result in a testing regime that would not only require no harm to animals but would increase the robustness of the testing regime thus reducing risk to people.

Executive Summary

This submission has new information not included in the submission sent to the health select committee on the Psychoactive Substances Bill, those familiar with the previous submission provided by NZAVS may wish to focus on pages five to nine. The new information is that

- the interim Psychoactive Substances Expert Advisory Committee (iPSEAC) was not aware that exposure to humans is an integral part of the development of psychoactive substances for the market
- iPSEAC agreed with significant aspects of the NZAVS expert advice and needs more time to investigate in detail the areas it did not immediately agree with
    - points in agreement
        - acute toxicity testing is not necessary
        - the LD50 test is outdated and should not be used
        - data from animal tests are not good predictors of human response
    - points of contention
        - that a suitable number of validated non-animal tests are available
        - that the agreed problems with the extrapolation of animal data to humans are best dealt with by more animal tests rather than other methods

As no information is available on what areas the government believes animal testing is unavoidable we are unable to focus information on available non-animal tests where it is most needed. Due to this unfortunate lack of information from iPSEAC the rest of this

submission includes material already submitted to the health select committee and not considered. It does not though include those sections where iPSEAC has reached conclusions in line with the expert advice to NZAVS; we have removed the sections where there is no debate from the body of the submission though appendix one remains unaltered. The main points here are that

- animal tests are not the best predictors of drug responses in humans and are of limited value as pre-clinical tests (iPSEAC acknowledged there are problems this area)
- there are many non-animal tests available for the pre-clinical trials that are to be required before the planned human trials
- there is considerable public opposition to the use of animal tests for recreational drugs, the petition request would address that opposition
- the UK Home Office have confirmed that when similar legislation is passed in the UK no animal testing will be allowed to occur

Introduction

This submission from NZAVS will show that the use of animal tests in the pre-clinical stage of a testing regime is unnecessary and undesirable as such tests have been superseded by technological advances since the protocols for the animal tests were developed. Our aim is to show that putting in place legislation ensuring animal testing of recreational drugs does not occur will not compromise the effectiveness of the safety testing regime and, in fact, may enhance it.

The self-evident ethical issues with using animals for testing nonessential recreational products alone should be enough to require that the safety testing does not include the use of animal tests. We have been advised by toxicology experts, both internationally and locally based, that if directed by the policy makers to formulate a testing regime that does not use animals they could do so. The New Zealand public clearly desires that their representatives in Parliament provide this guidance[1]. We don't believe there is any argument against doing so that would outweigh these concerns.

The source for the recommendations of animal testing came from one report provided to the Ministry of Health (MoH) in its original version on 16th January 2012 and revised at different times during discussions between the author and the MoH until the final version was submitted on 21st March 2012. This report has the title "*Regulations Governing the Control of Novel Psychoactive Drugs – defining parameter associated with toxicity*" and was tabled in parliament so is not provided in full in this submission despite being referred to. A pdf of the report is also freely available online here: http://www.leaveanimalsout.org.nz/psychoactives%20testing%20-%20testing%20regime%20proposal%20report.pdf. The limited amount of information on advances made in recent decades in this report is of concern, as is the lack of options of possible testing regimes provided for policy makers and the complete lack of consideration

[1] "Only 14.8% of adult New Zealanders surveyed support allowing animal testing on psychoactive substances, like party pills, if it produces the best results" http://www.horizonpoll.co.nz/page/306/firm-no-to-party-pill-testing-on-animals

to the ethical issues relating to using animals for testing recreational drugs. We aim to fill in some of those omissions with this submission.

Appendix one of this submission contains a report written by two American toxicology experts provided to NZAVS and given to the MoH in 2012. This report outlines their concerns with relying on animal testing for safety testing. For each of the proposed testing types – acute toxicity, repeat dose toxicity, genotoxicity and toxicokinetics – the limitations and scientific concerns with animal tests are discussed and then available non-animal tests are listed and detailed. This report will be referred to and summarised in the body of the submission.

Two commonly used terms:

IN VITRO – experimentation using methods that don't require a whole living organism. More precisely this term doesn't include the use of isolated cells from donors or cell cultures and experimentation using these is correctly referred to as ***ex vivo*** but we follow the standard convention of including these cases under the term *in vitro.*

IN VIVO – experimentation using a whole living organism

New Information not included in the NZAVS submission on the Psychoactive Substances Bill

This section acknowledges the work done by iPSEAC in the limited amount of time they had available. The amount of time given to consider such a considerable amount of evidence on this important issue was limited. That iPSEAC only met to discuss this issue on 31 May 2013 when Hon Pater Dunne announced his intention to have an expert committee consider the evidence in early December 2012 is puzzling. It clearly shows iPSEAC did not have anywhere near the time it should have had to report in any kind of conclusive manner. Given the necessary time and clear guidance from legislators to find a suitable testing regime that does not use animals they will be able to do so.

We implore the government to give the permanent expert committee that time before sanctioning the use of animal tests. We also see it as vital that a process is put in place so that international toxicology experts are able to provide assistance if information is lacking in any area. New Zealand has no internationally recognised experts on non-animal testing. There are though such experts in Europe and the USA that are eager to assist in this process here. These people have provided information to regulators in their own countries and others that has been instrumental in allowing legislators to avoid requiring animal testing. All available information needs to be considered objectively, transparently and in a fair manner for there to be public confidence in the regulatory regime.

Not allowing the use of animal test data would not introduce any additional risks to people. It would not compel the regulatory body to approve any substances that it was not comfortable were of a low risk.

Exposure to Humans is part of the development process for recreational drugs

Controlled exposure to a group of human volunteers is done early in the development stage for psychoactive drugs; such human exposure is never done in the early development of pharmaceuticals. This is done in order to gauge the type of effect the drug will have on users before being developed into a consumer friendly product. This stage of the development process also means that any drugs that have an immediate severe adverse effect in humans do not go any further in to development.

In considering the pre-clinical testing iPSEAC did not consider that evidence of controlled human exposure would be available. NZAVS has been advised by iPSEAC member Bob Kerridge that the committee was not aware of this significant point of difference in the development of pharmaceuticals and recreational drugs. Their considerations were done under the assumption that there would be no prior human exposure. As the majority of the pre-clinical testing is done in order to establish an acceptable level of risk for controlled exposure to people in the human trials evidence of such prior human exposure would negate the need for a significant amount of the pre-clinical testing, animal or otherwise.

The recommendation to allow animal testing was not made with consideration of how psychoactive substances are developed. It was made by iPSEAC under the assumption that they were developed in a similar process as pharmaceuticals. This is not the case. Psychoactives substances are developed differently for a different purpose. While we agree with the expert advice we received, and included in appendix one, that many suitable and more reliable non-animal tests exist and should be used it is disappointing and concerning that these differences were not considered by iPSEAC. Full consideration of all the information may well have led to a different conclusion from iPSEAC.

The interim Psychoactive Substances Expert Advisory Committee's agreement with the expert advice from NZAVS

The initial advice received by the MoH recommending animal testing proposed the use of animals for acute toxicity testing. The advice received from NZAVS recommended that this area of testing not be carried out as studies have shown it to be of little use for safety testing (appendix one page 28). The interim Psychoactive Substances Expert Advisory Committee reached the same conclusion and recommended that acute toxicity testing not be required. Despite this the sections on acute toxicity testing are still included in the appendix. This has been done so the select committee has access to all the information and as some of the tests in this section are also of use for repeat dose toxicity testing.

The advice to the MoH recommending animal tests also included the use of the LD50 test despite its use in pharmaceuticals being withdrawn in 2002. The use of the LD50 test was quickly ruled out the Minister when it became public knowledge that it was being considered. The fact its use was suggested to the MoH by Leo Schep, deemed an acceptable part of a testing regime by now iPSEAC member Paul Fitzmaurice when he read Schep's advice to the MoH in 2012 and was then included in a MoH discussion document as part of the data set to be required by the government is highly concerning.

The advice the reliance on animal testing was based on did not appear to have any recognition of any recent developments in the field. The LD50 test, a test that hasn't been used for over ten years, was proposed and acute toxicity testing was considered necessary despite being found to be little values for regulatory use.

Animal testing for the toxicokinetic investigations was recommended despite the in vitro Caco-2 cell test having all but replaced animal tests internationally in pharmaceutical testing as it is a considerably better predictor of human drug metabolism.

Availability of non-animal tests

We have provided information on seven different tests with OECD regulatory approval, three that are in regulatory use by Health Canada and more that are recommended for regulatory use in the USA and used by the European Medicines Agency. There is also information on many other non-animal tests that have been in common use for ten to twenty years in both pharmaceutical development internationally and the safety testing of tobacco in the UK since their ban on all animal testing of tobacco products in 1997.

We acknowledge that some of the tests included in this submission have yet to be validated for regulatory use but they are not in the majority. Non-validated tests are included in order to give a full picture of the range of tests available and with the expectation that the regulatory body here would look at validating some for use in the approval process. To this end we provided all contact information for the companies that produce these tests. We are under the understanding that no contact has been made with any of these companies asking for more information on the tests they have available and what progress is being made towards having them validated. We have also been told that information that may allow the tests to be accepted for the purposes of the legislation is available had it been asked for.

The initial advice from iPSEAC was that the lack of validation is what is stopping them from ruling out animal testing. At no point though has any information been made available about what types of tests they believe this is a problem for. The advice we have received from international experts in regulatory toxicology testing and non-animal tests is that there should not be an issue here as there are validated and commonly used tests available for all the proposed tests. A suitable test battery for every area of testing that does not use animals is an option.

Due to the lack of detail available on where the perceived problems are we have been left with little option than to include detailed information on all possible tests; and have done so. The remainder of this submission addresses this and we apologise for its length but we

see no way of reduced the content without leaving out information that may be vital. To risk leaving out information that may be of use would be to do an injustice to the tens of thousands of people that supported the petition and to the health select committee which should have as full a picture as possible of the information available.

What follows has already been made available and will be familiar to those that have read the previous submission. Sections outlining concerns around animal tests that have been addressed by the inclusion of Clause 11A have also been removed in the interests of keeping the length to a minimum.

Why a Legislative Restriction on Animal Testing Should be Included in the Psychoactive Substances Bill

To allow easy alignment with similar legislation when it is introduced overseas

The UK has not allowed the safety testing of tobacco and alcohol since 1997 when a nationwide prohibition came into place following legislation from 10 years earlier. When Dr Williams, Chair of the National Animal Ethics Advisory Committee (NAEAC), queried the UK Home Office about this ban and the ban's possible application to other recreational drugs the Head of the Animals in Science Regulation Unit replied unequivocally "*The same would apply to any recreational drugs if the proposal was to safety test them as recreational drugs*"[2] (their emphasis). If a Bill similar to the Psychoactive Substances Bill is introduced in the UK the use of animal testing in the safety testing would not be allowed from the outset. If animal testing is allowed here this would put the NZ Government in the position of having instituted a testing regime allowing animal testing and then having legislation introduced elsewhere modelled on the NZ example that would exclude animal testing.

Other European countries including Belgium and Germany have also banned testing tobacco on animals and given the recent European Union (EU) ban on all animal testing of cosmetics, and the import and sale of any cosmetics tested on animals anywhere in the world, it is likely that any new legislation introduced in the EU regulating non-medicinal drugs would also reflect this move away from animal testing.

[2] Emails reproduced in full in Appendix Three.

To increase and uphold our global reputation as a nation that holds animal welfare in high regard

This legislation is of high profile overseas and its development and implementation is being watched by all manner of international bodies. The possible use of animal testing as part of the safety testing has been receiving international attention. By taking a principled stand now on the ethics of using animal testing for nonessential recreational drugs our reputation as a nation that values animal welfare will be reinforced and remain intact into the future. The lack of similar legislation overseas allows the New Zealand government to take a leading position in the testing of these drugs. There are no pre-existing regulatory regimes to align with that require animal testing of recreational drugs. New Zealand is in the enviable position of being able to say "*yes these drugs need to be regulated and shown to be safe but we will not do so at cost to animals*."

If we do not rule out the use of animal testing in the safety testing now and similar legislation is introduced overseas that does not allow animal testing (as any such legislation in the UK automatically would) it will reflect badly on New Zealand.

Ethical considerations

The ethical concerns around the use of animals for research, testing and teaching are considerable and need to be given consideration whenever the use of animals is a possibility. A cost-benefit analysis needs to be done prior to any animal use as covered by Section 80(1)(b) in part six of the Animal Welfare Act 1999. We are concerned that the ethical issues have not been given any consideration when the proposed testing regime was being drawn up. On 24 October 2012 NZAVS made an OIA request to the office of Hon Peter Dunne asking for copies of "*Any discussions, or advice that was sought by your office or the MoH, in the development of the proposed regime for testing psychoactive substances on the ethical and legal issues relating to using animals, and getting approval for using animals, in*

the testing of recreational drugs for human consumption". This request was unable to be fulfilled as no such information existed. We were assured in the response to that request that work on this needed to be done and would be done and we were thanked for bringing the issue to their attention.

We then repeated this request on 13 February 2013 to find out what progress had been made and to get an idea of the government's possible position on this issue. We also asked specifically for any communications with the NAEAC on this as we had been told the MoH would be approaching them about this as the NAEAC would be directly involved in the ethics approval process. We were eventually told on 23 April 2013 that any such information still did not exist and no such discussions had occurred.

That the ethical issues with using animals, which are clearly of considerable public and moral concern, were never considered in the Bill's development is worrying. Had this been considered from the outset we believe a clear direction away from the use of animals would have been made when the proposal for a workable testing regime was in the early stages. This would have avoided the entire issue coming up at this late stage. We are thankful though that it is not too late to address these significant concerns.

Such concerns around the testing of nonessential drugs have been addressed and cost-benefit analyses comparing the cost to animals and the benefit to society has been done overseas. The UK government's nationwide prohibition on the use of animals for testing alcohol or tobacco products that came into effect in 1997 was done out of ethical concerns. The Home Office stated that, in making a cost-benefit analysis, it could not justify the use of animals, classifying these experiments as "morally or ethically objectionable"[3]. Additionally, the internationally renowned UK Nuffield Council on Bioethics reported that the Home Office "issued a policy statement to the effect that, in making the cost-benefit assessment,

[3] Home Office. *The Cost/Benefit Assessment, Chapter 2, Annexes 1-3, Appendix F, Report of the Animal Procedures Committee for 1997 (London: TSO)*, <http://www.rspca.org.uk/ImageLocator/LocateAsset?asset=document&assetId=1232712104105&mode=prd > (1997).

these tests were no longer considered a sufficient benefit to justify any use of animals."[4] We believe that the evidence available indicating public opinion on this issue shows that the NZ voters want our Government to follow that lead.

To reflect public opinion and ensure the legislation is as uncontroversial as possible and has the maximum public support

Public opinion on the use of animal testing in this case is clear; it is overwhelmingly against it happening. A recent Horizon poll found that only 14.7% of those polled thought animal testing should be allowed ***if*** it was the best testing method available. This result was so strong that Horizon used the headline "***Firm "no" to party pill testing on animals***" on their website when reporting the findings[5]. This poll result echoed earlier polls such as the NZ Herald Digi-poll survey that found 73.7% of those polled did not want any animal testing of recreational drugs[6]. Legislation should ideally, when possible, reflect the will of the voters and in this instance there is little debate about what the voters of New Zealand want and for the legislation to reflect what the voters' desire is possible.

Any legislation, such as this Bill, that seeks to regulate and allow the sale of recreational drugs that are used by a small minority of the population and are considered by a significant portion of voters to be undesirable and of detriment to society is going to be controversial. As well as this there is the fact that the Bill may require the use of animal testing that is opposed by approximately 85% of the population. We believe that a legislative restriction on the use of any animal testing data would allow this Bill to have a much public support as possible and would make it as uncontroversial as possible. As this bill is a positive move towards reducing the harm from the unregulated sale of recreational drugs we believe that removing the issue of animal testing will benefit the Psychoactive Substances Bill greatly.

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[4] Nuffield Council on Bioethics. *"Ethics of Research Involving Animals," Chapter 13.30*, <http://www.nuffieldbioethics.org/sites/default/files/The%20ethics%20of%20research%20involving%20animals%20-%20full%20report.pdf> (2005).

[5] http://www.horizonpoll.co.nz/page/306/firm-no-to-party-pill-testing-on-animals

[6] The New Zealand Herald. *"Kiwis oppose animal trials for party pills"* 31 Dec 2012. http://www.nzherald.co.nz/nz/news/article.cfm?c_id=1&objectid=10856707

Limitations of Animal Based Tests

Here the main points showing the limitations of animal tests will be covered with the details and other references available in Appendix One.

Animal models are of little use for investigating inhalation toxicity as it is not possible to make laboratory animals inhale the products being tested in the same way that humans will. Also the physical and physiological differences in the respiratory systems are significant, making extrapolation of the data difficult. This is evidenced by chronic cigarette studies in rats, mice, hamsters, dogs and non-human primates that do not show the significant increases in tumour development that occur in human smokers. This is, as a tobacco industry consultant recently wrote, "clearly at variance with the epidemiological evidence in smokers"[7].

Academic reviews of rodent tests to predict toxicity in humans have shown they are only about 40-60% predictive[8,9]. The various modern tests using cell lines give results that are 80-97% predictive, e.g. for liver 80%[10] and heart 97%[11]

Animals have significantly different metabolisms and physiology to humans. As a result, before *in vitro* ADME (absorption, distribution, metabolism, and excretion) studies on human cell models were routinely used by the pharmaceutical industry, the failure rate of

[7] Coggins, C. An updated review of inhalation studies with cigarette smoke in laboratory animals. *Int J Toxicol* 26, 331-338 (2007)

[8] Olson, H. *et al.* Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regulatory toxicology and pharmacology : RTP* 32, 56-67, doi:10.1006/rtph.2000.1399 (2000).

[9] Spanhaak, S., Cook, D., Barnes, J. & Reynolds, J. *Species Concordance for Liver Injury From the Safety Intelligence Program Board*, <http://bioblog.instem.com/downloads/SIP_Board_Species_Concordance.pdf> (2008).

[10] O'Brien, P. J. *et al.* High concordance of drug-induced human hepatotoxicity with *in vitro* cytotoxicity measured in a novel cell-based model using high content screening. *Archives of toxicology* 80, 580-604, doi:10.1007/s00204-006-0091-3 (2006).

[11] Inoue, T., Tanaka, K., Mishima, M. & Watanabe, K. Predictive *in vitro* cardiotoxicity and hepatotoxicity screening system using neonatal rat heart cells and rat hepatocytes. *AATEX* 14, 457-462 (2007).

drugs in clinical trials due to poor prediction of ADME was 40% - now it is only 10%[12]. This decrease in the failure rate shows that the modern *in vitro* tests are suitable predictors of human response. Due to the increase in prediction rate animal testing is no longer the generally accepted way of doing these tests in the development of new pharmaceuticals.

Animal testing for toxicology is no longer international standard practice. In 2008 in the United States of America the Environmental Protection Agency, the National Institutes of Health and the Food and Drug Administration announced a joint plan to replace all toxicology testing on animals with more modern techniques using human cells and human proteins as the use of these methods instead of animal models "will generate more data relevant to humans"[13]. This system is now in place and producing data[14].

[12] McKim, J. M., Jr. Building a tiered approach to *in vitro* predictive toxicity screening: a focus on assays *with in vivo* relevance. *Combinatorial chemistry & high throughput screening* 13, 188-206 (2010).
[13] Greenemeier, L,; Feds Agree to Toxicity Tests That Cut Animal Testing. *Scientific American;* February 15, 2008.
[14] Biello, D.; Robot Allows High Speed Testing of Chemicals. *Scientific American*; October 13, 2011.

Available Non-Animal Tests

The expert report contained in Appendix One lists multiple non-animal tests that are available and that the authors recommend as suitable for the testing of recreational drugs under the Psychoactive Substances Bill. Detailed comments on each of the testing methods listed below are provided in the report, as are references for more information and for contacting the manufacturers of the tests (where suitable). This section shows that there are a number of non-animal tests available that were not considered by the MoH in the development of this Bill when animal testing was considered, and should be considered now.

The report in Appendix One, while containing information on a number of suitable tests for every step of the proposed testing regime, is not a comprehensive list of such tests. Following the summary of the information in Appendix One is a list of further tests that should also be considered.

Repeat Dose Toxicity

- Ames Test – Bacterial Reverse Mutation Assay
- Neutral Red Uptake Assay
- In Vitro Micronucleus Assay
- Various Neutral Red Uptake Assays as already approved by the OECD and recommended in the US by ICCVAM[15]

[15] The Interagency Coordinating Committee on the Validation of Alternative Methods; part of the US National Institute of Environmental Health Sciences' National Toxicology program, ICCVAM is an interagency committee composed of representatives from 15 U.S. Federal regulatory and research agencies that require, use, generate, or disseminate toxicological and safety testing information used to determine the safety or potential adverse health effects of chemicals and products to which workers and consumers may be exposed.

- Various *in vitro* cell line studies using cells from the liver, kidneys, heart, nerves, lungs, bone marrow etc.
- QSAR models

The report authors note that the first three listed here are the only toxicity tests required by the Canadian Government for tobacco[16] and say that those tests, in the context of the pre-clinical trials, "*…should be adequate to assess the safety of smoked psychoactive drugs.*"[17]

Toxicokinetic Investigations

- PRIT Air/Liquid Interface culture and exposure system – for pulmonary absorption of inhaled substances
- Various *in vitro* dermal absorption tests
- Intestinal absorption in Caco-2 cells – for internal absorption of orally administered substances
- Liver-on-a-chip – for modeling metabolism in the human liver. (This is noted as especially important due to the poor correlation between animal models and human metabolism and liver toxicity).
- Physiologically based toxicokinetic (PBTK) models – for modelling distribution and excretion of substances through the human body; derived from existing data from *in vivo* or *in vitro* assays
- *In vitro* assays on hepatocytes – freshly isolated or cultured liver cells can be used to study possible metabolites and metabolism in a target organ

[16] See the Health Canada website http://www.hc-sc.gc.ca/hc-ps/tobac-tabac/legislation/reg/indust/method/tox-eng.php and copies of the methods for the tests are available on request to TRR_RRRT@hc-sc.gc.ca but aren't included in the appendix here as they total 53 pages and are freely available and provided in a short time frame directly from Health Canada. NZAVS can also supply copies on request to members of the Select Committee if it is desired.

[17] Appendix One, page 24.

- *In vitro* assays in conjunction with physiologically based pharmacokinetic (PBPK) modeling – as found by Pfizer to give the best predicative data when compared to animal models

Genotoxicity/Carcinogenicity

The following *in vitro* genotoxicity tests **all** have regulatory acceptance by the OECD and can be used to indicate the possible genotoxicity of the substance being tested. It is worth noting that two of the three tests in the test battery initially recommended to the MoH were *in vitro* tests. The replacement of the

- Bacterial reverse mutation (Ames) test
- *In vitro* cell gene mutation test in mammalian cells (MLA)
- *In vitro* chromosomal aberration test in mammalian cells (CA)
- *In vitro* mammalian cell micronucleus test (MNT)

The full report in the appendix also lists more tests that are currently undergoing the process of gaining validation.

Developmental and Reproductive Toxicity

NZAVS has the understanding that developmental toxicity tests are not likely to occur but in case they are to be required the report in the appendix contains lists of available non-animal tests for possible testing requirements: embryonic development, male and female fertility and endocrine effects.

Other references to available non-animal tests

Other options than those listed above and discussed in detail and referenced in Appendix One are available. Some examples include:

- Tagged microdosing – ultralow doses of the test drugs are tagged with a marker molecule before being administered to humans in highly controlled trials. This allows the toxicokinetic properties to be found in a way that is much more accurate than animal trials.[18]
- Since the UK ban on animal testing for the safety testing of tobacco British American Tobacco (BAT) have developed *in vitro* tests that are used to find the amount of particulate material in smoke, inflammation stress on the lungs and tests examining the risks of cancer, pulmonary disease and cardiovascular disease. They have an open access policy on their scientific findings and publish them in peer reviewed journals globally. Information about their latest developments in these areas and links to manuscripts of the published papers that can all be freely downloaded and viewed without a subscription can be found here at http://www.bat-science.com under "In vitro methods" in the "Science" section.
- There is an industry group called the In Vitro Testing Industrial Platform (IVTIP) that specialises in the use of in vitro testing for regulatory and safety testing. They are a global group of companies and test developers and would be able to provide information on the many non-animal toxicology tests available that would be suited for the requirements of the proposed legislation. More information can be found at http://www.ivtip.org

[18] Barnes, K. *New data adds weight to case for microdosing*. Outsourcing Pharma website, 24 June 2008. http://www.outsourcing-pharma.com/Preclinical-Research/New-data-adds-weight-to-case-for-microdosing

Extra Points to Consider

Known hazards of smoke inhalation

No matter what the active psychoactive ingredient, any smokable substance will contain known carcinogens and other toxins. There is enough existing data showing this to justify not giving an approval for sale to any smokable substance. Doing this prior to the pre-clinical testing will preclude a lot of testing that would be redundant as the results can be easily predicted from existing data. We recommend that this be done and note that it was indicated to NZAVS in discussions with the manufacturers that something along these lines in the legislation or regulations was expected by them.

Recreational drugs aren't medicines

Psychoactive drugs aren't medicines; the testing regime doesn't need to be aligned with medicines but should be modelled on other recreational drugs or unessential items people choose to use. No one has to take psychoactive drugs in order to remain healthy and alive. People choose to take them, they are to be age restricted, the sale controlled and daily use is not recommended. A better comparison than medicines would be to legal recreational drugs like alcohol and tobacco or to cosmetics. Cosmetics that are applied to the skin aren't regulated as if they are medical ointments as they aren't required for medical reasons yet they can contain potentially harmful ingredients. Likewise recreational drugs shouldn't be treated as medicines as they aren't required for medical reasons either.

There are many examples of countries that have banned the toxicity testing of the recreational drugs alcohol and tobacco on animals. The UK put this into law in 1987 with the ban coming into place in 1997 and other European countries have followed suit.

The MoH itself acknowledges the differences between the drugs covered by this Bill and pharmaceuticals and expected the toxicological data package to differ from that for pharmaceuticals. Among the reasons given by a MoH official in emails obtained under the OIA are the lack of a health claim, their use being discouraged and advised against and that the use will be less frequent than for pharmaceuticals[19]. We agree with that assessment and believe those factors need to be considered when establishing the testing regime and that they show that the establishment of a unique testing regime that excludes animals is possible and desirable.

[19] See Appendix Two for an example email discussing this.

Model Testing Regime

Any model testing regime will utilise a battery of tests, rather than relying on any one test, to provide enough information for any regulatory body to make a decision. Using a battery of *in vitro* tests before proceeding on to human trials that are closely monitored and use microdosing is common practice now. This model is enshrined in UK legislation for the trialling of cancer drugs and is one that is suitable for the requirements of the Bill being considered here.

The model shown below in Figure One is a general example put forward by Dr Andrew Knight, European Veterinary Specialist in Welfare Science, Ethics and Law and Fellow of the Oxford Centre for Animal Ethics. Dr Knight has expressed considerable interest in the proposed use of animal testing for recreational drugs and was only unable to make a submission himself due to the short time period available for submissions. It is a model that would provide a more than cautious enough approach for the required toxicological testing.

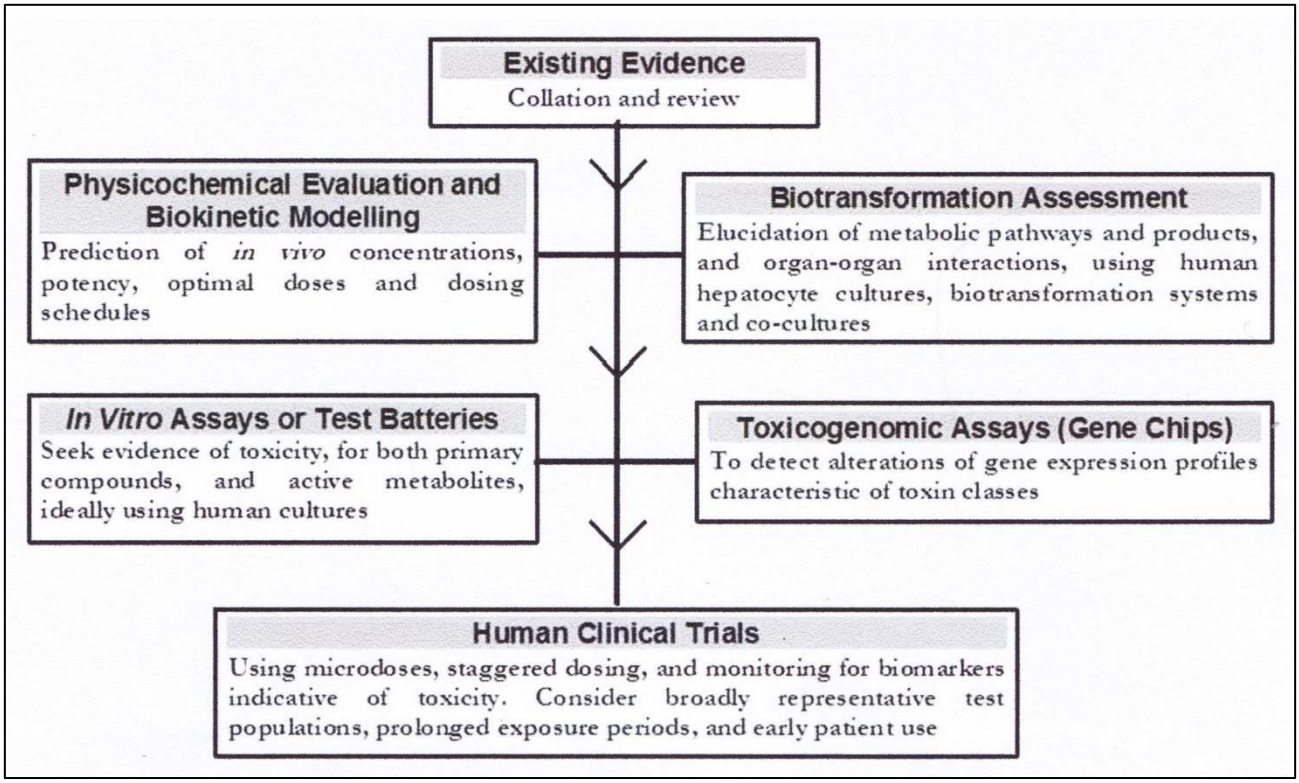

Figure One: An integrated toxicity testing model utilising *in vitro* methods for the pre-clinical trial stage

Conclusion

Public opposition to any animal testing of the recreational drugs covered by the Psychoactive Substances Bill is considerable and clear. The use of animals in the testing of nonessential recreational drugs is unethical. Toxicology experts have stated that non-animal tests are available and a suitable testing regime can be developed that does not use animal testing. It is up to legislators to provide them with clear guidance to do so. There is no barrier to introducing a legislative ban on the admissibility of data from animal tests. Such a ban should be included in the legislation to ensure the concerns of the New Zealand voters are addressed.

We wish to thank the health select committee for their time in considering this submission and look forward to answering questions in person and reading of the Health Select Committee's findings and recommendations to Parliament on the use of animals for the testing to be required by the Bill.

Appendices

Appendix One

Introduction

The advice that follows in the report "*Comments on 'Regulations Governing the Control of Novel Psychoactive Drugs – Defining Parameters Associated with Toxicity*'" was written by Amy Clippinger Ph.D. and Kirstie Sullivan M.P.H. Amy has a Ph.D. in cellular and molecular biology and genetics and several years of research experience at the University of Pennsylvania and Kirstie received her Master of Public Health in Toxicology in 2003 from the School of Public Health at the University of Michigan in Ann Arbor. Her studies included environmental health exposure and risk assessment, pharmacology, carcinogenesis and environmental diseases, and pathology.

This report was prepared in response to the document "*Regulations Governing the Control of Novel Psychoactive Drugs – defining parameters associated with toxicity*" that was provided to the Ministry of Health in its final version on 21 March 2012 and obtained by NZAVS from the office of Hon Peter Dunne under the Official Information Act 1982 on 10 October 2012. The document "*Regulations Governing…*" was sent to Kirstie Sullivan for her opinion. NZAVS was then supplied with the following report that was immediately passed on to officials at the Ministry of Health for their information.

Comments on "Regulations Governing the Control of Novel Psychoactive Drugs – Defining Parameters Associated with Toxicity"

We are very concerned that in its current form, the *Regulations Governing the Control of Novel Psychoactive Drugs – Defining Parameters Associated with Toxicity* ("The Regulations") would lead to unnecessary animal studies being conducted. While it is stated in the Executive Summary that these drugs will be tested "in a similar process to that required for pharmaceutical drugs," the results of drug studies in animals are often not predictive for human health and can lead to dangerous drug reactions in humans (as will be described in more detail below). We recommend that non-animal testing methods be used exclusively to evaluate the health risks of these drugs.

In their current version, The Regulations require chemistry, manufacturing and controls information, preclinical toxicology studies, human clinical studies and post-registration surveillance. Required preclinical toxicology studies include acute and repeated dose toxicity testing, toxicokinetic, carcinogenicity, genotoxicity and developmental studies in animals.

We support the recommendation that chemistry, manufacturing and controls information of constituents should be the first step in the evaluation of any new product. Drugs should also be compared to similar drugs for which toxicity data already exists[1].

In vitro evaluation of products should precede controlled human *in vivo* assays. *In vitro* preclinical assays will identify particularly risky or toxic products that should not be tested in humans. At this point, prior to commencement of human studies, applications to test novel psychoactive drugs should be made publicly available for comments by interested persons.

When used in this manner, laboratory analysis and *in vitro* preclinical assays will be sufficient to predict particularly toxic drugs and preclude the use of animal tests. Clinical work and post-registration surveillance will always be required to unequivocally determine the safety of a drug in humans and to examine mental effects associated with use, such as dependence and withdrawal. As stated in Section 4.0 "Human Clinical Studies" of The Regulations, "*animal testing does not always predict performance in humans* and cannot therefore guarantee the safety of the drug in humans" – further supporting the elimination of any animal tests. For any products whose safety cannot be determined by non-animal tests alone, the ethical implications of inducing suffering and death in animals in the name of recreational drug use should be seriously considered.

Acute and Repeated Dose Toxicity

Psychoactive drugs may be ingested or smoked; therefore, the method of assessing toxicity may differ depending on the intended route of human exposure.

Inhalation Toxicity

Scientifically, numerous obstacles exist in gathering human-relevant results from animal tests designed to assess products that are inhaled (that is, herbs or substances that are smoked). First, it is not possible to make laboratory animals use products that are inhaled the way humans do. Second, inherent interspecies differences prevent meaningful extrapolation of animal results to humans. The respiratory system in humans is quite different physically and physiologically than the respiratory systems in the most commonly-used test species, rats and mice.

In this regard, studies with combusted tobacco products have shown that chronic bronchitis cannot be replicated in rodents and that the data are inconsistent as to whether inhaled tobacco smoke can induce tumors and cancers in animal models. A recent article written by a tobacco industry consultant reported that results from years of chronic cigarette inhalation studies in rats, mice, hamsters, dogs, and nonhuman primates do not show significant increases in tumor development and are "clearly at variance with the epidemiological evidence in smokers, and it is difficult to reconcile this major difference between observational studies in humans and controlled laboratory studies in five different species."[2] The major reasons for these discrepancies are the fundamental physical, metabolic, and physiological differences between animals and humans, especially with regard to respiratory anatomy and physiology.[3] In the same regard, psychoactive drugs that are smoked will face similar issues with interspecies extrapolation.

Given the physical and physiological differences and the methodological challenges presented by attempting to replicate the human smoking experience in animals, the human relevance of the data collected from animals in this realm is negligible and the suffering imposed on these animals unjustifiable.

Non-Animal Methods to Assess Acute Inhalation Toxicity

In vitro alternatives exist to assess inhalation toxicity of smoked products, including the reconstructed human tissue models described in Table 1. Tobacco industry scientists have concluded that "*in vitro* toxicology tests can be successfully used both for better understanding the biological activity of cigarette smoke… and for guiding the development of cigarettes with reduced toxicity."[4] Thus, these methods can and should also be applicable to novel psychoactive drugs that are smoked.

As an example, the Canadian government's federal *Regulations Amending the Tobacco Reporting Regulations* requires that manufacturers conduct three tests to assess the toxicity of their tobacco products. All of the required tests are *in vitro*, non-animal methods – bacterial reverse mutation assay, neutral red uptake assay, and the *in vitro* micronucleus assay[5]. These tests are widely validated and have been shown to effectively identify the mutagenicity, cytotoxicity, and

clastogenicity, respectively, of whole cigarette smoke as well as individual tobacco ingredients and compounds. If the Canadian government deems these *in vitro* tests sufficient to analyze the toxicity of cigarettes, they should be adequate to assess the safety of smoked psychoactive drugs.

Method Name	**Comments**
MatTek's (Ashland, Mass.) EpiAirway™ System	Consists of normal, human-derived tracheal/bronchial epithelial (NHBE or TBE) cells that have been cultured to form a pseudo-stratified, highly differentiated three-dimensional model closely resembling the epithelial tissue of the respiratory tract. EpiAirway tissues are grown on cell culture inserts at the air-liquid interface, allowing for gas phase exposure of volatile materials in airway inflammation and irritancy studies, as well as in inhalation toxicity studies. This system has been well characterized histologically and biochemically (cell markers) and in terms of biological response to known toxins and pharmaceuticals. See: http://www.mattek.com/pages/products/epiairway
SkinEthic Laboratories' (Nice, France) reconstructed human esophageal and alveolar epithelium models	These models use immortalized human esophageal (Kyse 510) or alveolar (A549) cells and are structurally and mechanically similar to MatTek's and also form epithelial tissue that histologically resembles cell layers of the human lung. See: http://www.skinethic.com/index.asp
Epithelix's (Genève, Switzerland) MucilAir	Epithelix's MucilAir is a three-dimensional model of the human airway epithelium which is made of primary human cells isolated from the nasal cavity, the trachea and the bronchus. This model mimics the *in vivo* tissues of the human respiratory epithelium. This model can also be used for repeated-dose studies because the cells maintain their characteristics for up to a year in culture. See: http://www.epithelix.com/content/view/5/6/lang,en/
Wyss Institute's Lung-on-a-chip	Lung-on-a-chip mimics the complicated mechanical and biochemical behaviors of a human lung. See: http://wyss.harvard.edu/viewpage/240/

Oral Toxicity

It is clear that the results of acute toxicity testing in animals are not relevant to human health considerations. The European Medicines Agency (EMEA) Committee for Medicinal Products for Human Use (CHMP) "Concept paper on single dose acute toxicity" presents the findings of a joint information-sharing initiative among 18 European pharmaceutical companies and contract research organizations seeking to establish the relevance of acute toxicity data to the drug development process[6]. In a striking consensus, these companies agreed that single dose acute toxicity studies are not useful for keeping unsafe compounds from reaching human trials and do not provide unique insights into the safety of a possible medicine. Additionally, a coalition of European pharmaceutical companies determined that regulatory decisions were almost never predicated on the results of acute oral toxicity tests[7], prompting the removal of the requirement for acute toxicity testing from the International Council on Harmonization (ICH) M3 guidelines for non-clinical safety studies for human clinical trials of pharmaceuticals[8].

With regard to whether acute toxicity testing is useful to predict the consequences of human overdose, Chapman *et al* report a consensus among representatives from poison centers, the pharmaceutical and chemical industries, and regulatory bodies that the information it provides is of little value[9]. This is partly because high doses of chemical substances often elicit non-specific effects in animals that have no relevance to incidences of human overdose. In addition, acute toxicity testing typically does not provide information on adverse and functional effects, target organ toxicity, and toxicokinetics that is considered by poison centers to be most useful. The authors conclude that better information for the treatment of poisoning could be obtained from tests that are already carried out as part of the regulatory process.

Furthermore, hazard classification often does not adequately predict human toxicity[9]. A study of outcomes of human poisoning cases with three organophosphorous pesticides, all categorized as class 2 (LD_{50} > 5 ≤ 50 mg/kg) by the Globally Harmonized System of Classification and Labelling of Chemicals, found significant differences in severity of symptoms and likelihood of death, despite having similar LD_{50} values from acute toxicity studies[10]. Even in cases for which hazard class has been reported to correlate with mortality, mortality rates are highly variable among substances within a class; in one study, mortality rates for seven compounds in class 1 ranged from 24% to 0%[11].

Non-Animal Methods to Assess Acute Oral Toxicity

There are several alternatives to oral toxicity testing in animals, including the Normal Human Keratinocyte Neutral Red Uptake (NHK NRU) Assay, the Balb/c 3T3 Neutral Red Uptake (3T3 NRU) Assay, the EvaTOX assay (currently awaiting acceptance from ECVAM to enter their validation programme), and Quantitative Structure-Activity Relationship (SAR) Models (Table 2). The results for immortalized 3T3 cells and primary NHK cells were similar in the validation study; however, the 3T3 NRU assay is more cost and time effective than the NHK NRU assay[12].

The United States Environmental Protection Agency (EPA) affirms that data from the 3T3 Neutral Red Uptake (NRU) cytotoxicity assay may be used in a weight-of-evidence approach for determining

starting doses for *in vivo* acute oral systemic toxicity studies but not for hazard category classification purposes. Recently, the ACuteTox project reported the results of its prevalidation of a tiered testing strategy using eight *in vitro* assays[13]. The outcome of this study reinforced previous results obtained with the 3T3 NRU assay, supporting its use to identify unclassified substances (LD_{50} > 2000 mg/kg) as a first step in a tiered testing strategy. In addition, a number of assays were identified that were able to flag substances as neurotoxicants and nephrotoxicants. These assays could be used to alert on tissue-specific toxicity for substances that are identified as toxic (predicted LD_{50} < 2000 mg/kg) with the 3T3 NRU assay. It was also concluded that the combined use of DEREK and METEOR software is likely to improve the ability to predict the toxicity of an unknown substance or its major metabolites.

TABLE 2: Non-Animal Methods to Assess Acute Oral Toxicity

Method Name	**Acceptance**	**Comments**
Balb/c 3T3 Neutral Red Uptake (3T3 NRU) Assay	OECD GD 129 (2010) and recommended to U.S. agencies by ICCVAM (2008) to estimate starting doses for oral acute toxicity	**Principle of the Test:** The NRU assays are based on the ability of viable cells to take-up and store the dye neutral red so that test substances that cause cell death and/or inhibition of cell growth will result in a decrease in the amount of neutral red retained by the culture. The *in vitro* 3T3 NRU cytotoxicity assay has been demonstrated to correctly discriminate non-toxic (those with an $LD_{50} \geq 2000$ mg/kg) from more toxic chemicals[14], and shows very good correlation with mammalian LD50 data at both extremes of the toxicity spectrum (i.e. very toxic and non-toxic)[13]. Both NRU *in vitro* assays (3T3 and NHK) are approved to determine starting doses of test substances for two acute oral toxicity test methods (the Up-and-Down Procedure OECD 425 and the Acute Toxic Class Method OECD TG 423). **Congruence with *in vivo* data:** 3 laboratories independently tested the ability of the 3T3 NRU assa distinguish between toxic and non-toxic chemicals with 56 chemicals and obtained 92-96% sensitivity[15]. **Considerations:** Limitations to both NRU *in vitro* assays (3T3 and NHK): General differences between cell culture systems and animals create a difference with respect to how a substance is delivered and how it is distributed and metabolized within cells. Because animals must absorb the substance after oral administration, certain organs may not be exposed to the same amount of the

		substance or may not be exposed to the substance for the same length of time; this is in contrast to the direct addition of the test substance to cells in culture. Additionally, if a test substance only produces toxicity through a specialized mechanism in a specific cell type, the effect may not be observed in 3T3 or NHK cells. 3T3 and NHK cells have little to no capacity to metabolize xenobiotic compounds.
Normal Human Keratinocyte Neutral Red Uptake (NHK NRU) Assay	OECD GD 129 (2010) and recommended to U.S. agencies by ICCVAM (2008) to estimate starting doses for oral acute toxicity	**Principle of the Test:** See principle of Balb/c 3T3 Neutral Red Uptake (3T3 NRU) **Considerations:** See considerations of Balb/c 3T3 Neutral Red Uptake (3T3 NRU)
CeeTox's AcuteOralTox-LD50 *in vitro* screen		A recent collaboration between CeeTox and L'Oreal has resulted in the development of an AcuteOralTox-LD50 *in vitro* screen which combines several *in vitro* concepts to predict acute oral toxicity without using animals[16]. This screen considers both pharmacological and physical-chemical properties of a substance in addition to the CTOX Panel®, which is a multi-parameter, cell-based *in vitro* system for predicting acute systemic toxicity. Analysis of 76 substances demonstrated that 75% of chemicals in GHS categories 1, 2 and 3 were correctly classified and the sensitivity and specificity were 85% and 89%, respectively, at an LD50 threshold of 500 mg/kg. L'Oreal has already assessed more than 100 compounds using this assay, and a manuscript on the assay and results is currently in the process of being submitted for publication.
MatTek's EpiOral and EpiGingival models		For oral toxicity testing, MatTek's EpiOral and EpiGingival models consist of normal, human-derived epithelial cells that allow *in vitro* study of irritation, oral pathologies, and basic oral cavity phenomena. The cells have been cultured to form multilayered, highly differentiated models of the human buccal (EpiOral) and gingival (EpiGingival) tissues. Morphologically, these tissue models closely parallel native human tissues, thus providing a useful *in vitro* means to assess irritancy, disease, and other basic oral biology phenomena. These tissue models have been extensively studied. SkinEthic also offers models of reconstructed human oral and gingival epithelium. These cell systems have been well characterized in terms of histology, biochemistry, and biological response.

		See: http://www.mattek.com/pages/products/epioral
Quantitative Structure-Activity Relationship (QSAR) Models		QSAR models can be used to estimate the likelihood of toxicity of chemicals (for example, the combined use of DEREK and METEOR software can be used to predict the toxicity of an unknown substance and its major metabolites).

Non-Animal Methods to Assess Repeated-Dose Oral Toxicity

Differences in the activities of the liver are a major contributor to the species differences observed in the toxicity of chemicals and drugs. Several reviews of the ability of rodent tests to predict human toxicity have shown that they are only about 40-60% predictive[17,18].

TABLE 3: Non-Animal Methods to Assess Repeated-Dose Oral Toxicity (adapted from [19-21])

Target	Method Name	Comments
Liver	*In vitro* hepatotoxicity on human liver cell lines	One study showed 80% of 243[22] and another showed 100% of ten[23] hepatotoxicants were detected using this method.
Kidneys	*In vitro* kidney cell lines	One study showed good prediction with *in vivo* data for 15 nephrotoxicants tested using this method[24].
Heart	*In vitro* heart cells	One study showed 81% of six[25] and another showed 97% of four[26] cardiotoxicants were detected using this method.
Nerves	*In vitro* neuronal cells test	Excellent agreement between *in vivo* and *in vitro* predictions for organophosphorus compounds[27].
Lungs	EpiAirway or MucilAir: *In vitro* lung epithelial cells	81% correlation with existing human data with 11 chemicals using MucilAir[28].
Immune System	CFU-GM (from bone marrow cells) *In vitro* human whole blood cytokine assay *In vitro* lymphocyte proliferation assay	Accurate prediction of *in vivo* results for five out of six substances tested for a pre-validation study; positive results for an additional 20 substances tested[29]. The *in vitro* results correlated well with *in vivo* data for 31 compounds tested[30]. 100% of 6 immunotoxic compounds were detected using this method[31].
QSAR Computer	TOPKAT	QSAR computer models can be used to assess repeated dose

Models	DEREK LAZAR	toxicity. TOPKAT was able to predict 30% LOAELs within a factor of 3, 60% within a factor of 10 and 96% within a factor of 100 for 393 chemicals tested[32]. LAZAR showed 89% accuracy within 1 log from experimental value[33].

Toxicokinetic Investigations

Animals have significantly different metabolism and physiology to humans. As a result, before *in vitro* ADME studies on human cell models were routinely used by the pharmaceutical industry, the failure rate of drugs in clinical trials due to poor prediction of ADME was 40% - now it is only 10%[34].

Non-Animal Methods to Assess Toxicokinetics

TABLE 4: Non-Animal Methods to Assess Toxicokinetics		
Endpoint	**Method Name**	**Comments**
Pulmonary Absorption (human lung epithelial for inhalation)	PRIT Air / Liquid Interface (ALI) culture and exposure system	The PRIT® ALI system uses membrane cultures of adherent cells or tissues and can be used to study inhalable substances. See: http://www.item.fraunhofer.de/de/forschungsbereiche/toxikologie-umwelthygiene/in-vitro-toxikologie/PRIT.html
Absorption	*In vitro* dermal absorption test	*In vitro* dermal absorption studies may provide information to characterize systemic absorption (through skin *or* other routes).
Absorption	Intestinal Absorption in Caco-2 cells	See: http://www.item.fraunhofer.de/de/forschungsbereiche/toxikologie-umwelthygiene/klinische-chemie.html
Distribution	Human-on-a-chip	Human-on-a-chip integrates multiple organ-on-a-chip systems to mimic the whole human body See: http://wyss.harvard.edu/viewpressrelease/91/
Metabolism	Liver-on-a-chip	The liver-on-a-chip is designed to mimic what happens in the human body and will be especially important considering how poorly animal studies predict human metabolism and human liver toxicity. See: http://spectrum.mit.edu/articles/features/liver-on-a-chip/
Distribution and Excretion	Mathematical physiologically-based toxicokinetic (PBTK) models	Mathematical physiologically-based toxicokinetic (PBTK) computer models consist of a set of physiological and chemical parameters that can predict the distribution and excretion of substances through the human body following initial input of information on absorption and metabolism. This information can be derived from existing *in vivo* or from *in vitro* assays. • 80% accurate distribution for 123 drugs within 2-fold error[35]. • 70% accurate for 19 drugs tested[36]. • 90% accurate prediction of renal excretion for 40 compounds tested[37]. • 88% precise prediction of renal clearance for 141 drugs

		tested[38].
Metabolism	*In vitro* assays on hepatocytes	Freshly isolated or cultured hepatocytes and subcellular fractions (e.g. microsomes) from liver may be used to study possible metabolites and examine local metabolism in a target organ. It may be useful to study the inhibition and induction of specific cytochrome P450 isozymes (e.g., CYP1A1, 2E1, 1A2, and others) and/or phase II enzymes by the parent compound using *in vitro* studies. Information obtained may have utility for similarly structured compounds[39]. A review of studies showed that hepatic clearance could be predicted using human liver microsomes[40]. *In vitro* assays using human liver cells were as predictive as animal tests for 50 drugs tested[41]. *In vitro* tests with PBPK modeling (SCHH-PBPK) were more accurate for humans than *in vivo* rat and dog assays[42]. Also see: http://www.item.fraunhofer.de/de/forschungsbereiche/toxikologie-umwelthygiene/klinische-chemie.html for examples: CYP profiling (microsomes), CYP inhibition screening (microsomes), CYP induction (primary human hepatocytes), N-acetyltransferase profiling (microsomes)

Genotoxicity and Carcinogenicity

The 2-year cancer bioassay in rodents has poor concordance between species (for example between humans and rats or rats and mice). Poor interspecies extrapolation can result from a number of different reasons, for example, different tumor types and mechanisms which are of little or no relevance to humans (described in more detail in PETA 2007[43]). This test is generally known to have serious limitations in its ability to predict human cancer risk[44,45]. In Europe, the most commonly performed carcinogenicity tests are the lifetime rodent bioassay[46] and combined chronic toxicity/lifetime rodent bioassay[47]. However, little attempt has been made to validate the lifetime rodent bioassay against human carcinogenicity[48]. According to Ennever *et al*[49], the sensitivity of animal bioassays is very high (all definite human carcinogens adequately tested were positive); however, the specificity is low. A survey of the US Environmental Protection Agency database to assess the human utility of animal carcinogenicity data showed the animal data were predictive for 42% of chemicals[44].

In 2006, People for the Ethical Treatment of Animals, US, (PETA US) analyzed the first 500 rodent cancer assays conducted by the US National Cancer Institute and National Toxicology Program (NTP) and found that these agencies judged approximately one in every seven studies to produce either equivocal evidence of carcinogenic activity or to be scientifically inadequate[43]. PETA US also analyzed the ability of one species/gender group (e.g., male mice) to predict the cancer risk for other groups of rodents (e.g., female rats) exposed to the same chemical and found that results in one species and gender frequently underestimated cancer incidence in the other species and genders, with the average false negative rate being 27.5 percent, but ranging as high as 40 percent in one case. With regard to false positives, the NTP has acknowledged that about half the chemicals it has tested have produced evidence of cancer in rodents[50-52] and reported that "two-thirds of the positive bioassays were positive only when the [maximum tolerated dose] was employed." The maximum tolerated dose is the highest-dose of a substance that will not shorten the animals' normal life span because of noncancer-related toxic effects and is often several orders of magnitude greater than typical environmental exposures. At these doses, cancer may result from nonspecific mechanisms such as increased cell proliferation.

Non-Animal Methods to Assess Carcinogenicity and Genotoxicity

Replacement of *in vivo* carcinogenicity testing can be achieved by employing a range of tests that assess both genotoxic and non-genotoxic effects. Table 5 describes the *in vitro* genotoxic assays that are accepted by the OECD and cell transformation assays (CTAs) that are in the process of OECD acceptance.

A number of well-established and regulatory-accepted *in vitro* genotoxic tests are available. Kirkland *et al* demonstrated that 93% of 553 rodent carcinogens were detected in at least one of the three most common *in vitro* genotoxicity tests (Ames-test, mouse lymphoma Assay and the *in vitro* micronucleus Test or Chromosomal Aberration Test)[53]. However, a caveat to the use of these tests is the relatively low specificity and high rate of misleading positive results, especially for tests measuring clastogenic effects (breaks in chromosomes, leading to sections of the chromosome being deleted, added, or rearranged) 20. The combination of three *in vitro* genotoxicity tests as required

by the European Scientific Committee on Consumer Safety (SCCS) increases the sensitivity of the test battery (up to 90%), but the specificity (ability to identify non-carcinogens) decreased to below 25%.

Cell transformation assays (CTA) can detect both genotoxic and non-genotoxic carcinogens. These assays have been in use since the 1960s but have only recently been considered for regulatory use. CTAs rely on changes in cell colony morphology and monolayer focus formation. The CTAs are currently used for confirmation of *in vitro* positive results from genotoxicity assays and can be used in the weight of evidence assessment. Data generated by CTAs can also be useful where genotoxicity data for a certain substance class have limited predictive capacity (e.g. aromatic amines), for investigation of compounds with structural alerts for carcinogenicity or to demonstrate differences or similarities across a chemical category[20]. In addition, the tumor-promoting activity of chemicals can be investigated by the CTA.

The use of non-testing methods, including (quantitative) structure-activity relationships ([Q]SARs), grouping and read-across are an attractive means of filling data gaps in both hazard and risk assessment without requiring additional testing. (Q)SARs are mainly used for screening but also provide a means of filling data gaps in hazard assessment. Adler *et al* describe the status of (Q)SARs for carcinogenicity testing[20]. Most models are qualitative (SARs) and QSARs for non-genotoxic carcinogenicity are still in an early stage of development. Several (Q)SARs are available for predicting genotoxicity and carcinogenicity[54]. Freely available models in the public domain include CAESAR, Toxtree, OncoLogic, LAZAR and the OECD QSAR Toolbox. Commercial models requiring license fees include MultiCase, TOPKAT, HazardExpert, DEREK and ToxBoxes.

The threshold of toxicological concern (TTC) is a statistical approach used to establish a conservative default risk value based on worst-case assumptions about the chemical in the absence of data. It has regulatory acceptance as a risk assessment tool in the US for food packaging material and in the US and Europe to set acceptable exposure limits for genotoxic impurities in drugs. It has not yet been granted regulatory acceptance for use in cosmetics in Europe although the SCCS is conducting an on-going evaluation of the use of TTC for cosmetics.

Taylor *et al* describe an integrated testing strategy that combines the exposure-based threshold of toxicological concern approaches, with OECD accepted *in vitro* genotoxicity tests and CTA assays to replace *in vivo* carcinogenicity studies and provide a precautionary approach for consumers[19]. If the human exposure exceeds the TTC levels the Ames test, and one other genotoxicity test, should be performed. If both are positive it should be assumed that the chemical is a genotoxic chemical; if there is any doubt a CTA assay should be performed. Benigni and Bossa demonstrated that a tiered testing strategy, with inexpensive and fast tests in Tier 1 (e.g. the Ames test or structural alerts) and the Syrian Hamster Embryo (SHE) CTA in Tier 2, is able to identify up to 90% of carcinogens[55].

British American Tobacco uses the following *in vitro* assays to measure the cytotoxicity and genotoxicity of extracts of smokeless tobacco or the particulate phase of combustible tobacco smoke components: (1) the Ames test, to measure effects on single DNA bases (gene mutations) in bacterial cells; (2) the *in vitro* micronucleus assay, to measure structural and numerical changes to chromosomes in mammalian cells; (3) the *in vitro* mouse lymphoma assay, to measure gene mutations and chromosome aberrations in mammalian cells; and (4) the Neutral Red cytotoxicity

assay, to measure cellular viability[56]. The genotoxicity assays (Ames assay, *in vitro* micronucleus assay and *in vitro* mouse lymphoma assay) measure the ability of the chemicals to cause changes at different levels of the genetic material and each assay has different sensitivities (as discussed below). Therefore, when all three genotoxicity assays are combined, together with the cytotoxicity assay, they are able to detect most mutagens and cytotoxic compounds. The Committee on Mutagenicity (COM) "Guidance on a strategy for Testing of Chemicals for Mutagenicity"[57] currently recommends the Ames test, the *in vitro* micronucleus assay and the *in vitro* mouse lymphoma assay for the *in vitro* testing of chemicals. The Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) *in vitro* toxicology task force[58] recommends the Ames test and the neutral red uptake assay. Therefore, the battery of *in vitro* assays used by British American Tobacco meets the requirements of both COM and the CORESTA *in vitro* toxicology task force.

Table 5. *In vitro* genotoxicity and cell transformation assays that can be used in an integrated testing strategy to replace *in vivo* carcinogenicity studies (adapted from [19-21])

Method	**Regulatory Acceptance / Status of validation**	**Comments**
Genotoxicity Tests	OECD TG 471 (1997): Bacterial reverse mutation (Ames) test	**Principle of the test:** Identifies gene mutations (point mutations, base pair substitutions and frame shift mutations). **Congruence with *in vivo* data:** • 90% of rodent carcinogens detected when combined with MLA and MNT assays[53] • 77% accuracy on 368 chemicals[59] • The application of the Ames test to a large number of chemicals has shown that this test has a high positive predictivity for chemical carcinogens (around 80%)[60]. **Considerations:** Prokaryotic cells differ from mammalian cells in factors such as uptake, metabolism, chromosome structure and DNA repair processes. *In vitro* tests often require the use of an exogenous source of metabolic activation which cannot mimic entirely the mammalian *in vivo* conditions. The test may not be appropriate for the evaluation of certain classes of chemicals, for example highly bactericidal compounds and those which are thought (or known) to interfere specifically with the mammalian cell replication system (e.g. some topoisomerase inhibitors and some nucleoside analogues). For a full list of considerations see OECD 471.
	OECD TG 476 (1997): *In vitro* cell gene mutation test in mammalian cells (MLA)	**Principle of the test:** Identifies gene mutations (point mutations, base pair substitutions and frame shift mutations) and structural and numerical chromosome damage in Mouse Lymphoma L5178Y cells. **Congruence with *in vivo* data:** • 90% of 553 rodent carcinogens detected when combined with MNT and Ames test[53]

		Considerations: See comments on Ames test regarding metabolism. False positive results may arise from changes in pH, osmolality or high levels of cytotoxicity when the test chemical is added to the medium. Assay does not detect carcinogens that act by non-genotoxic mechanisms. The assay may have low specificity. For a full list of considerations see OECD 476.
	OECD TG 473 (1997): *In vitro* chromosomal aberration test in mammalian cells (CA)	**Principle of the test:** Identifies structural and numerical chromosome damage in mammalian cells (i.e. clastogenicity and polyploidy) **Congruence with *in vivo* data:** • 85% of 553 rodent carcinogens detected when combined with Ames test and MLA32 **Considerations:** See comments on MLA. For a full list of considerations see OECD 473.
	OECD TG 487 (2010): *In vitro* mammalian cell micronucleus test (MNT)	**Principle of the test:** Identifies structural and numerical chromosome damage in mammalian cells (i.e. clastogenicity and aneuploidy) **Congruence with *in vivo*:** • 83% agreement on 113 chemicals in ECVAM validation study[61] **Considerations:** See comments on MLA. For a full list of considerations see OECD 487.
Cell Transformation Assays (to detect genotoxic and non-genotoxic carcinogenicity)	Syrian Hamster Embryo (SHE) pH 6.7 and pH 7 (OECD TG in preparation) Balb/c 373 (Currently undergoing validation by ECVAM) Bhas 42 (Validation ongoing by JaCVAM)	**Principle of the test:** Used for screening, clarification of *in vitro* genotoxic positive results, hazard identification, identification of promoters, chemopreventative activity and mechanistic studies. Exposure to carcinogenic chemicals results in an increase of morphologically transformed colonies, which are characterized by disorganized growth patterns and considered as an early stage in the carcinogenic process **Congruence with *in vivo*:** • The SHE (pH ≥7 and pH 6.7) correctly identified 100% of the 44 inorganic human carcinogens tested and identified 9 out of 11 organic carcinogens[62]. A meta-analysis performed by the OECD indicated that the three CTA assays have an overall sensitivity of 90% of class I (known) and 95% of class II (possible/ probable) human carcinogens[62] . • SHE has a concordance with the rodent bioassay ranging from 85% (SHE pH ≥7) to 74% (SHE pH 6.7)[63]. • ECVAM workshop found that 80-83% rodent carcinogens were detected on 213 chemicals[64]. • P&G study showed 85% agreement with rodent data with 56 chemicals[65]. • Pfizer study showed 89% agreement with rodent data with 19 chemicals[66]. **Considerations:** SHE cells retain the ability to biotransform

		xenobiotics. Future developments: An improved protocol has been developed for the Balb/c 3T3 method which allowed more reproducible results to be obtained. It should also be noted that the SHE assay uses embryos harvested from hamsters that are killed for this purpose. **Status:** A prevalidation study with SHE (pH 6.7 and 7.0) was organized by ECVAM to address issues of standardization of the protocols, transferability and reproducibility. The experimental work finished in 2009. The data demonstrated that the SHE protocols and the assay system themselves are transferable between labs.

Developmental and Reproductive Toxicity

Animal tests for reproductive toxicity take a long time and use many animals. In addition, a number of studies have shown that they only detect about 60% of known human reproductive toxicants[67,68]. The EU ReProTect project concluded that a battery of *in vitro* tests "allowed a robust prediction of adverse effects on fertility and embryonic development"[69], with a combined accuracy of 70 to 100% for ten test chemicals[70].

TABLE 6: Non-Animal Methods to Assess Developmental and Reproductive Toxicity (adapted from [19-21])

Endpoint	Test Method	Comments
Embryonic development	*Ex vivo* whole embryo culture test (WEC) Micromass test (MM)	An ECVAM validation study showed up to 80% accuracy with 14 chemicals (100% for strong embryotoxicants)[71].
	Mouse/human embryonic stem cell test (EST)	An ECVAM validation study showed 78% agreement for 14 chemicals (100% for strong embryotoxicants)[71]; another study showed 75% agreement with *in vivo* for 63 chemicals[72]; another study showed 88% accuracy for eight drugs[73].
Male fertility	Computer-Assisted Sperm Analysis (CASA)	This test was evaluated by two laboratories with more than 35 chemicals[70].
	Testicular fragment culture	82% correlation with *in vivo* data for 11 chemicals tested[74].
	Leydig cell test	Good correlation for 15 chemicals[70] and detected 100% of five endocrine disruptors[75].
	Sertoli cell test	Good correlation for seven chemicals in two different laboratories[70].
Female Fertility	Bovine *in vitro* (oocyte) maturation (bIVM)	Good correlation with *in vivo* results for 15 chemicals[76] and good correlation on eight chemicals when tested in different laboratories[77].
Endocrine Effects	Estrogen receptor alpha binding assay	High accuracy for ranking 12 chemicals as strong, weak or no effect[78].
	Estrogen receptor (ER) – transcriptional activation assay, MELN	High accuracy on 16 chemicals and good inter-laboratory concordance[79].
	AR CALUX reporter gene assay	74% agreement on an inter-laboratory study of 64 chemicals[80]; excellent agreement for 14 out of 16 in a pre-validation study[81]; 85% agreement with the animals test for 50 chemicals[82].

	Estrogen receptor transcriptional assay, LUMICELL-ER	100% of 28 estrogen receptors were detected[83].
	OECD TG 455[84]: Stably transfected transcriptional activation assay (STTA) estrogen	80% accuracy for 46 chemicals tested[85].
	H295R steroidogenesis assays based on a human cell line	78% accuracy for testosterone effect on 18 chemicals, 88% for estradiol effect on 16 chemicals[86].

Conclusion

In 1997, the U.K. government enacted a nationwide prohibition on the use of animals for testing alcohol or tobacco products[87]. The Home Office stated that in making a cost-benefit analysis, it could not justify the use of animals, classifying these experiments as "morally or ethically objectionable".[88] Additionally, the internationally renowned UK Nuffield Council on Bioethics reported that the Home Office "issued a policy statement to the effect that, in making the cost-benefit assessment, these tests were no longer considered a sufficient benefit to justify any use of animals."[89]

In addition to ethical or economic considerations, the use of animals to determine the safety of novel psychoactive drugs is scientifically unjustified. Important differences in the anatomy and physiology between humans and other animals make relying on animal tests to predict human safety dangerous. And, as described above, there are numerous non-animal tests that meet or exceed the accuracy of animal tests for predicting human health hazards. The Canadian government only requires *in vitro* tests to assess the safety of tobacco products[5]. In a similar manner, the safety of novel psychoactive drugs can be determined using a battery of *in vitro* tests. Thus, for scientific, economic and ethical reasons, the use of animals for testing psychoactive drugs is indefensible and we hope that the government will use its authority to ensure that only human-relevant and humane non-animal testing methods will be utilized to assess the risks of these products and fulfill the data submission requirements.

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Appendix Two

Email correspondence from MoH Senior Policy Analyst Mark Heffernan to Dr Paul Fitzmaurice at ESR; released to NZAVS under the OIA.

Printed by Mark Heffernan

Sent by: Mark Heffernan/MOH

18/01/2012 04:01 p.m.

To: Paul Fitzmaurice <Paul.Fitzmaurice@esr.cri.nz>
cc:
bcc:

Subject: RE: Criteria for assessing the risk of harm from new psychoactive substances

Thanks, Paul

Yes, please do forward a copy on to Keith!

Indeed, the tox data package required for designer drugs is likely differ from that of pharmaceuticals for a number of reasons. For example, not only will designer drugs not be making a health claim as do pharmaceuticals, their use will continue to be discouraged and advised against which is not generally the case for pharmaceuticals. Secondly, use of designer drugs will arguably be less frequent that of pharmaceuticals which may require testing to ascertain the safety of their daily use.

Your initial feedback is useful and appreciated! I look forward to discussing this report with your further when you return.

Cheers

Mark Heffernan
Senior Policy Analyst
Sector and Services Policy
Ministry of Health
DDI: (04) 8163392

http://www.moh.govt.nz
mailto:Mark_Heffernan@moh.govt.nz

Paul Fitzmaurice Hi Mark, I will take this documen... 18/01/2012 03:54:28 p.m.

From: Paul Fitzmaurice <Paul.Fitzmaurice@esr.cri.nz>
To: '"Mark_Heffernan@moh.govt.nz"' <Mark_Heffernan@moh.govt.nz>
Date: 18/01/2012 03:54 p.m.
Subject: RE: Criteria for assessing the risk of harm from new psychoactive substances

Redacted under Section 9(2)(a)

Hi Mark,

I will take this document on holiday we me to review. I have already done one read through and [redacted] has done a good job of summarising the main aspects of a standard Tox data package. However, given our previous chat, I am unsure if MoH would require every aspect of this package for a designer drug. Indeed you could argue that that level of testing would put these drugs in the Medicine/pharmaceutical bracket.

Would it be OK for me to send Keith a copy for his comments?

Cheers

Paul

From: Mark_Heffernan@moh.govt.nz [mailto:Mark_Heffernan@moh.govt.nz]
Sent: Wednesday, 18 January 2012 10:49 a.m.
To: Paul Fitzmaurice

Appendix Three

Email correspondence from NAEAC Independent Chairperson Dr Virginia Williams and Judy MacArthur Clark, Head of the Animals in Science Regulation Unit at the UK Home Office; released to NZAVS under the OIA.

------ Forwarded Message
From: Judy MacArthur Clark 9(2)(a)
Date: Mon, 18 Mar 2013 19:49:35 -0000
To: Virginia Williams 9(2)(a)
Cc: Judy MacArthur Clark <Judy.MacarthurClark@homeoffice.gsi.gov.uk>, Sue Houlton <sue.houlton@homeoffice.gsi.gov.uk>
Subject: RE: Animal testing

I'm sorry for the delay. I've been checking up the absolute legal status of our 'ban'.

In effect, our ban on safety testing tobacco and alcohol is applied through our project licensing system. Any proposal to test the safety of these substances would be mandatorily referred by inspectors upwards to higher management and would be rejected. We monitor this through our annual statistics which specifically ask whether procedures have been done to test the safety of tobacco products or alcohol. We have had nil returns in these categories since the 1990's.

The same would apply to any recreational drugs if the proposal was to safety test them as recreational drugs. However many of them have been safety tested for medicinal purposes (e.g. ketamine) and that is acceptable.

The other slight complication is that a number of procedures are authorised where animals will be exposed to tobacco (e.g. to induce smoking related lung pathology) or to alcohol (e.g. as a model of addiction or depression) but wherein the purpose is not to test the safety of the tobacco or alcohol product but rather to prepare a model of disease. After careful consideration, we will authorise appropriate applications for project licences along these lines. The same could apply to use of a recreational drug if it's use was to prepare a model of say schizophrenia. The annual statistics will not report such procedures as testing of tobacco or alcohol or recreational drug products since they are merely being used to create a disease model for other study.

I hope this is reasonably clear. Do come back to me if you have any queries.

All the best.

Judy

Judy MacArthur Clark CBE MRCVS

9(2)(a)

From: Virginia Williams
Sent: 13 March 2013 02:08
To: Judy MacArthur Clark
Subject: Re: Animal testing

Hi Judy

Do you have any update on this issue for me? Would be much appreciated.

On 4/03/13 3:52 AM, "Judy MacArthur Clark"

9(2)(a)

I'm just checking my draft response to your question below with my inspectors - just to ensure I understand accurately the current situation. Hope to have a confirmed answer shortly.

Judy

Judy MacArthur Clark CBE MRCVS

9(2)(a)

From: Virginia Williams 9(2)(a)
Sent: 01 March 2013 00:37

To: Judy MacArthur Clark
Subject: Animal testing

A question. I have been told that using animals in research into recreational drugs – cigarettes, alcohol etc – is not allowed in Britain. If this is so, can you please send me/direct me towards relevant information? We have a bill coming into Parliament that will require any "party pills" containing psychoactive substances to be tested for safety, with current suggestions to use rats to test for acute toxicity, repeat dose toxicity, pharmacokinetics and genotoxicity , before, depending on the results, going on to human trials.

9(2)(g)(i) Am also interested in finding any evidence on trials (human or animal) on the testing of such substances.

I do hope you can help or point me in the right direction.

------ End of Forwarded Message