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ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

**REPORT OF THE OECD/EFSA WORKSHOP ON DEVELOPMENTAL NEUROTOXICITY (DNT):
THE USE OF NON-ANIMAL TEST METHODS FOR REGULATORY PURPOSES**

Series on Testing and Assessment
No. 261



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OECD Environment, Health and Safety Publications

Series on Testing & Assessment

No. 261

**REPORT OF THE OECD/EFSA WORKSHOP ON DEVELOPMENTAL NEUROTOXICITY
(DNT): THE USE OF NON-ANIMAL TEST METHODS FOR REGULATORY PURPOSES**

IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

**Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris, 2017**

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FOREWORD

In November 2015, the European Food Safety Authority (EFSA) proposed a collaboration with the OECD to explore the possibility to establish a battery of in vitro assays for the investigation of developmental neurotoxicity (DNT) potential of chemicals. A project proposal was submitted by EFSA to the Test Guidelines Programme for the organisation of a joint international workshop. This report, agreed by the co-chairs, summarises the discussions and outcomes of the joint OECD/EFSA workshop on DNT, held on 18-19 October, 2016, in Brussels, Belgium.

This document is being published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology, which has agreed that it be declassified and made available to the public.

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INTRODUCTION

1. The overall goal of the workshop was the identification and possible uses of non-animal developmental neurotoxicity (DNT) test methods for the regulatory purpose of screening and testing of chemicals).
2. The workshop was organised to engage in discussions with experts on the regulatory need for alternative DNT methods, the suitability of a proposed battery of in vitro assays for screening chemicals, and recommend actions to facilitate the regulatory use and acceptance of these alternative test methods.
3. Fifty-nine experts from fifteen OECD countries, the European Commission, representatives of Business and Industry Advisory Committee to the OECD (BIAC), Industry representatives from ECPA (EU) and Crop Life America and research institutes/universities as well as the International Council for Animal Protection in OECD Programmes (ICAPO) participated in the workshop. The list of participants is available in [Annex 3](#). The workshop was co-chaired by Ellen Fritsche (IUF – Leibniz Research Institute for Environmental Medicine, Dusseldorf, Germany) and Kevin Crofton (US-EPA).
4. The workshop participants' conclusions and recommendations are included in the first part of this report. The workshop background document is presented in [Annex 1](#). The workshop programme is available in [Annex 2](#). The abstracts of presentations are compiled in [Annex 4](#), while presentations are provided in [Annex 5](#).

SETTING THE SCENE

5. DNT has been regarded as an area in need of methodological approaches to better detect and characterise hazardous chemicals, with a critical need for time- and cost- efficient predictive in vitro testing methods. A series of workshops held over the past decade have fostered the development of in vitro assays that assess the impact of chemicals on cellular processes critical to normal brain development, including: neural proliferation, differentiation, migration, neurite outgrowth, synaptogenesis, and neural activity ([CAAT DNT](#) workshops and [ISTNET](#) workshop). Many of these assays have been used to derive mechanistic information for limited numbers of chemicals. Very few have been used to screen large numbers of chemicals.
6. These datasets and the mechanistic information that derive from in vitro DNT assays can be fit for purpose to address specific regulatory needs such as:
 - a. The screening and prioritization (for further testing) of many chemicals used in commerce for which there is no data at all on their potential to cause DNT.
 - b. The inclusion of DNT in vitro data as part of the overall weight of evidence (WoE) in an integrated approach to testing and assessment (IATA).
7. Fit for purpose means that the use of in vitro DNT data for screening and prioritization may imply a broader acceptance of uncertainty in the assays and resulting data compared to the use of the battery in chemical specific assessments. The latter will require a higher degree of validation due to regulatory implications that may restrict chemical production and use, while the former will only suggest that further testing may be required. Thus, the level of validation required will depend on the expected use of the battery in the regulatory context, i.e. screening purposes versus hazard identification and risk assessment.
8. In order to accelerate the development and facilitate regulatory use of such tools, the workshop was co-organized by EFSA and the OECD. For EFSA, the current test paradigm is not sufficient for an

adequate assessment of pesticidal active substances. In Europe, for pesticidal active substances, specific DNT studies are not required a priori. However, when indicated by observations in other studies or the mode of action of the test substance, supplementary studies or information may be required to provide information on the postnatal manifestation of effects such as developmental neurotoxicity (EC Reg. 287/2013). Indeed, testing of DNT using OECD TG 443 (Extended One-Generation Reproductive Toxicity Study) or 426 (Developmental Neurotoxicity Study) studies can be requested in this regulatory context when data from standard adult and/or reproductive toxicity studies indicate a possible concern for neurotoxicity. Further triggers can be used for the conduct of the OECD TG 443 in other regulatory settings, e.g. under REACH where disturbances of the thyroid or sex hormone system are suggested. However, these guideline studies are very resource intensive in terms of animal usage, time and overall cost and have been used only for a limited number of chemicals or pesticidal active substances. This highlights the pressing need for standardised alternative methodologies that can more rapidly and cost-effectively screen large numbers of chemicals for their potential to cause DNT or investigate mechanisms to provide information on human relevance.

9. The OECD Test Guidelines Programme has experience developing guidance for testing, including the use of in vitro methods as alternatives to animal bioassays, and developing integrated approaches to testing and assessment. It is recognised by users and regulators that the in vivo assay (OECD TG 426) is complex to implement and seldomly used; the endpoints included in the assay suffer from a lack of mechanistic basis and it is unsuitable for testing a larger number of chemicals. Therefore, it was relevant for EFSA and OECD to co-organise the event.

10. A background document was prepared (see [Annex 1](#)) to facilitate the discussion in the break-out sessions. This background document contains a comprehensive overview of available AOPs concerning DNT and also includes additional toxicity pathways related to DNT. Furthermore, it identifies the necessary neurodevelopmental processes that need to be addressed in non-animal test methods in order to inform potential DNT hazard. The background document also provides an evaluation of the level of readiness of the in vitro assays for DNT testing that were previously summarised in an EFSA's External scientific report and proposes potential testing strategies and outlines a framework for building a DNT testing battery based on assay readiness.

11. The workshop focused on the following, issues, such as:

- regulatory background and latest developments;
- experiences within OECD countries and industry;
- scientific advances in detecting and predicting DNT.

PARTICIPANTS

12. Participants attending the workshop included:

- regulators and evaluators from European countries, United States and Canada;
- invited experts from research institutes (academia); and
- invited experts from key stakeholder groups such as industry and relevant Non-Governmental Organisations.

A list of participants is provided in [Annex 3](#).

PURPOSE AND SCOPE OF THE WORKSHOP

13. The main objectives of the workshop were to:

a. Develop consensus on the composition of a testing battery of alternative DNT methods ready to be used now for the following purposes:

-Chemical screening for prioritization, and

-Hazard identification for specific chemical risk assessment

b. Depending on the level of readiness of the alternative test methods, identify the next steps to encourage the regulatory use of the alternative methods (e.g. more precise test method description; better definition of limitations and applicability domain; guidance and frameworks for defining “fit for purpose”; Test Guideline development) either individually or in combination.

c. Outline what could become an integrated approach to testing and assessment (IATA) for the purposes for screening and prioritization or for hazard assessment.

STRUCTURE OF THE WORKSHOP

14. The workshop programme is provided in [Annex 2](#).

15. Presentations were grouped under four sessions, as follows:

- Introduction, providing a history and path forward related to alternative test methods for DNT;
- Government risk assessors experience and perspectives regarding DNT data requirements and testing strategies based on alternative DNT assays;
- Industry's experience and perspectives regarding a DNT testing battery and strategies, based on alternative assays;
- Academic scientists' opinions and rationale for encouraging the use of a DNT testing battery based on non-animal test methods.

16. After each presentation a short question-and-answer session was held, with the opportunity for more discussion at the end of the workshop.

17. Following these general presentations, case studies illustrating potential testing strategies and outlining a framework for building a DNT testing battery were presented (see [Annex 1, section 5](#)). These case studies were used to facilitate the discussion during the break-out sessions that followed. Questions posed to the groups can be found in the workshop programme in [Annex 2](#). Following the discussions in the breakout sessions on the afternoon of the first day, rapporteurs of each group presented the outcome in plenary.

OUTCOMES AND RECOMMENDATIONS FOR POSSIBLE FURTHER WORK

18. Participants agreed that the presentations at the workshop had covered approaches and concerns from regulatory, industry and research perspectives. The two co-chairs summarised the outcomes and recommendations of the workshop as follows:

a) A consensus was achieved that current in vivo data requirements for DNT testing are considered insufficient for either screening and prioritization or hazard identification.

b) A consensus was reached that in vitro DNT data is considered useful in regulatory actions as a first screening step in prioritization or hazard identification.

A flexible in vitro testing battery should:

- allow prioritizing chemicals for further DNT testing
- be a prerequisite to conduct targeted in vivo testing in a tiered testing approach

c) The proposed draft in vitro testing battery to be used for the following regulatory purposes:

- immediately, for screening of chemicals and prioritization,
- followed by further harmonization in an international acceptance process through OECD, with an acknowledgement that improvements will continue to be made as science advances and regulatory acceptance increases and that changes in regulation i.e. data requirement, are needed.

d) An agreement was achieved on the need for a draft framework for regulatory use of DNT data through an integrated approach to testing and assessment (IATA). The framework should be driven by problem formulation as defined by decision-making needs. It should make efficient use of resources. As the potential impact of the regulatory decision increases, data needs and resources use will increase to reduce the scientific uncertainty in estimates of risk and impact.

e) It was acknowledged that a budget is critically needed from global stakeholders to undertake several of the tasks identified above, including:

- acceleration of testing large chemical libraries with hundreds of compounds
- annotation of the individual in vitro assays in the in vitro testing battery using OECD GD 211
- development of more reference chemicals for the generation of data using the in vitro testing battery
- generating data in support of the in vitro assays performance
- developing initial guidance for data interpretation via IATA

19. Other issues were raised in the course of the discussions that are also addressed in other fora, including:

- Exposure
- Biokinetics
- Metabolism
- Indirect pathways such as thyroid hormone-driven pathways
- Cumulative exposure.

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ANNEX 1 – BACKGROUND DOCUMENT

Please refer to the separate publication for full Annex 1

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ANNEX 2 - WORKSHOP PROGRAMME

Overall Chairperson | **Ellen Fritsche**, *IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf (DEU)*

Overall Rapporteur | **Kevin Crofton**, *U.S. Environmental Protection Agency (USA)*

DAY 1 | TUESDAY, 18 OCTOBER 2016

07.45-08.30

Registration

SESSION 1 | OPENING ADDRESS

08.30-08.50

Welcome and introduction to the event

Anne Gourmelon, *Organisation for Economic Co-operation and Development (OECD)*
Thorhallur Halldorsson, *University of Iceland on behalf of European Food Safety Authority (EFSA)*

08.50-09.20

Alternative Test Methods for Developmental Neurotoxicity: A History and Path Forward
Questions

Kevin Crofton, *U.S. Environmental Protection Agency (USA)*

SESSION 2 | What can be learned from regulatory authorities regarding DNT data requirements and testing strategies based on alternative DNT assays?

09.20-09.40

EU regulatory perspective with special focus on pesticides
Questions

Roland Solecki, *German Federal Institute for Risk Assessment (DEU)* & **Susanne Hougaard Bennekou**, *The Danish EPA (DNK)*

09.40-10.00

Developmental neurotoxicity under the REACH
Questions

Hannele Huuskonen, *European Chemicals Agency (ECHA)*

10.00-10.30

COFFEE / TEA BREAK

10.30-10.50

US regulatory perspective with special focus on pesticides
Questions

Elissa Reaves, *U.S. Environmental Protection Agency (USA)*

10.50-11.10

US Regulatory Perspective on Developmental Neurotoxicity Testing with Special Focus on Endocrine Disrupting and Industrial Chemicals
Questions

Stanley Barone Jr., *U.S. Environmental Protection Agency (USA)*

SESSION 3 | What can be learned from industry's experience with DNT testing strategies based on alternative assays?

11.10-11.30	EU industry perspective: Emphasis on Pesticides Questions	Gaby Schmuck representing <i>European Crop Protection Association (BEL)</i>
11.30-11.50	U.S. Industry Perspective: DNT Testing Strategies Based on Alternative Assays Questions	Sue Marty <i>DOW (USA)</i>

SESSION 4 | Why should we encourage the use of DNT testing strategies based on non-animal test methods?

11.50-12.10	Perspectives on how the Adverse Outcome Pathway (AOP) concept informs the use of <i>in vitro</i> DNT data for regulatory purposes Questions	Anna Price , <i>European Commission Joint Research Centre (JRC)</i>
12.10-12.30	How to link test systems to the prediction of developmental neurotoxicity (DNT) Questions	Marcel Leist , <i>University of Kostanz (DEU)</i>
12.30-13.30	LUNCH BREAK	

SESSION 5 | Discussion Group (DG) sessions: OECD case studies for potential testing strategies based on non-animal test methods and a draft framework for building a DNT testing battery

13:30—14:00	Introduction to OECD case studies for potential testing strategies and a draft framework for building a DNT testing battery Questions	Ellen Fritsche , <i>Dusseldorf University (DEU)</i>
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14:00-16:00

DG session 1 | The regulatory need

DG Chairperson | **Roland Solecki**, *German Federal Institute for Risk Assessment (DEU)*

DG Rapporteur | **Martin Wilks**, *University of Basel (CHE)*

1. Define a general DNT-based problem formulation for risk assessment of chemicals under the different regulations.
2. What is needed to achieve regulatory acceptance of alternative methods for DNT to be applied for screening and prioritisation?
3. What types of data from alternative DNT methodologies can be used to inform regulatory needs for hazards identification of different chemical classes?
4. How we can justify the need for a mandatory tiered approach (e.g. for specific classes of pesticides) for conducting *in vitro* and (targeted) *in vivo* DNT studies?
5. What input do scientists need from the risk assessors and risk managers to help guide development of

in vitro methods?

DG session 2 | Proposing a draft DNT testing battery

DG Chairperson | **Antonio Hernandez-Jerez**, *University of Granada School of Medicine (ESP)*

DG Rapporteur | **Anna Price**, *European Commission Joint Research Centre (JRC)*

Based on the background document provided, and different regulatory needs, recommend a testing battery based on alternative DNT methods considering the following items:

1. *What are the criteria for combining assays to create ITS/IATA for different regulatory purposes to identify compounds with DNT potential?*
2. *How to improve readiness and standardisation of available in vitro assays?*
3. *How AOP concept can assist assay selection for DNT testing?*
4. *How to overcome major limitations of alternative approaches?*

DG session 3 | How can knowledge from new DNT tests contribute to epidemiology and vice versa?

DG Chairperson | **Stanley Barone Jr.**, *U.S. Environmental Protection Agency (USA)*

DG Rapporteur | **Marcel Leist**, *University of Konstanz (DEU)*

How can in vitro methods contribute to the following issues:

1. *Building confidence in a scientific argument based on mode of action leading to adverse effects, supporting epidemiological observations (AOP and other tools)*
2. *Identification of DNT hazards from chemicals present in environmental samples (e.g. diesel exhaust)*
3. *Characterisation of the hazard level of suspected toxicants*
4. *Hazard ranking of related compounds – relation to exposure*
5. *Identification of potential toxic mechanisms*
6. *Identification of biomarkers for toxicants - relation to exposure (biomonitoring)*
7. *Comparison of species susceptibility*

DG session 4 | Implementing a draft DNT testing battery

DG Chairperson | **Susanne Hougaard Bennekou**, *The Danish EPA (DNK)*

DG Rapporteur | **Elissa Reaves**, *U.S. Environmental Protection Agency (USA)*

If the proposed draft testing battery is deemed adequate for considered use, then:

1. *What is necessary to apply the testing paradigm as a screening tool as a first step for DNT?*
2. *Depending on the intended use, which are the critical methodological gaps that can be identified?*
3. *What additional work is needed for the regulatory implementation of the methodologies in the evaluation of DNT-related hazards for single chemical entities or (relevant) chemical classes?*
 - a) *More in-vitro screening testing (i.e. define specificity and sensitivity)?*
 - b) *Mechanistic validation (i.e. transcriptome analysis, pathways)?*
 - c) *What are the appropriate steps of a road map?*
 - d) *Can regulators agree on this road map and how to implement it?*

16:00-16:30

COFFEE / TEA BREAK

16:00-18:00

ROOM | FOYER A (outside plenary room)

18.00-19.30

DG sessions: case studies continued

NETWORKING COCKTAIL

ROOM | O BAR

WEDNESDAY, 19 OCTOBER 2016

SESSION 6 | Reports from Discussion Group (DG) sessions

08.30-08:50

Report from DG session 1

Martin Wilks, *University of Basel (CHE)*

08.50-09.20	Report from DG session 2	Anna Price , <i>European Commission Joint Research Centre (JRC)</i>
09.20-09.40	Report from DG session 3	Marcel Leist , <i>University of Konstanz (DEU)</i>
09.40-10.00	Report from DG session 4	Elissa Reaves , <i>U.S. Environmental Protection Agency (USA)</i>
10.00-10.30	COFFEE / TEA BREAK	
10.30-12.00	Discussion on DG reports/outcomes	Kevin Crofton , <i>U.S. Environmental Protection Agency (USA)</i>
12.00-12.30	Conclusions and recommendations	Ellen Fritsche , <i>Dusseldorf University (DEU)</i> & Kevin Crofton , <i>U.S. Environmental Protection Agency (USA)</i>
12.30-13.00	Closing remarks	

ANNEX 3 - LIST OF PARTICIPANTS

**Participants list for OECD/EFSA Workshop on Developmental Neurotoxicity
(DNT): the use of non-animal test methods for regulatory purposes**

18-19/10/2016

LAST NAME	FIRST NAME	AFFILIATION	COUNTRY
ALI MOHAMMED	Ifthekhar	Swedish Chemical Agency (KEMI)	SWE
ARABI	Azadeh	Swedish Chemical Agency (KEMI)	SWE
BAILEY	Francis	Health Canada	CAN
BARENYS	Marta	Leibniz Research Institute for Environmental Medicine (IUF)	DEU
BARONE	Stanley	U.S. Environmental Protection Agency (EPA)	USA
BISSON	Michèle	Institut National de l'Environnement Industriel et des Risques (INERIS)	FRA
CALAMANDREI	Gemma	Istituto Superiore di Sanità (ISS)	ITA
CASTELAIN	Philippe	Scientific Institute of Public Health (WIV-ISP)	BEL
CROFTON	Kevin	U.S. Environmental Protection Agency (EPA)	USA
DEMENEIX	Barbara	CNRS MNHN	FRA
FABRIZI	Laura	European Commission	
FASSBENDER	Christopher	PETA International Science Consortium Ltd	UK
FRITSCHÉ	Ellen	Dusseldorf University	DEU
GALL	Andrea	Federal Institute for Risk Assessment (BfR)	DEU
GARCIA	Jesús Pablo	Institute of Health Carlos III	ESP
GARMENDIA	Irantzu	Fecc - European Association of Chemical Distributors	BEL
GOURMELON	Anne	Organisation for Economic Co-operation and Development (OECD)	
GOUZE	Marie-Estelle	French Agency for Food, Environmental and Occupational Health & Safety (ANSES)	FRA
HAKKERT	Betty C.	National Institute for Public Health and the Environment (RIVM)	NLD
HALLDORSSON	Thorhallur Ingi	University of Iceland	ISL
HENDRIKS	Hester	National Institute for Public Health and the Environment (RIVM)	NLD
HERNANDEZ-JEREZ	Antonio	University of Granada	ESP
HESEL	Ellen	National Institute for Public Health and the Environment (RIVM)	NLD
HOGBERG	Helena	Johns Hopkins University	USA
HOUGAARD BENNEKOU	Susanne	The Danish EPA	DNK
HUUSKONEN	Hannele	European Chemicals Agency	
HYNES	Jarlath	Humane Society International/Europe	BEL
IBANEZ	Matilde	Solvay	BEL
JACOBI	Sylvia	Albemarle Corporation	BEL
LEIST	Marcel	University of Konstanz	DEU

LAST NAME	FIRST NAME	AFFILIATION	COUNTRY
LIU	Zhiwei	FMC Corporation	USA
MARTY	Sue	DOW	USA
MASJOSTHUSMANN	Stefan	Leibniz Research Institute for Environmental Medicine (IUF)	DEU
MCHUGH	Damian	Philip Morris International	CHE
MEHL	Anna	Norwegian Food Safety Authority+	NOR
MORETTO	Angelo	Università degli Studi di Milano	ITA
MUNDY	Bill	U.S. Environmental Protection Agency (EPA)	USA
MUNN	Sharon	European Commission - DG Joint Research Centre (JRC)	
PARRA MORTE	Juan Manuel	European Food Safety Authority (EFSA)	
PISTOLLATO	Francesca	European Commission - DG Joint Research Centre (JRC)	
PRICE	Anna	European Commission - DG Joint Research Centre (JRC)	
REAVES	Mary Elissa	U.S. Environmental Protection Agency (EPA)	USA
REID	Kirsty	Eurogroup for Animals	BEL
ROSENTHAL	Esther	Federal Institute for Risk Assessment (BfR)	DEU
ROZNOVSKÁ	Zuzana	Research Institute for Organic Syntheses Inc (VUOS)	CZE
RUSCONI	Manuel	Federal Office of Public Health	CHE
SACHANA	Magdalini	Organisation for Economic Co-operation and Development (OECD)	
SCHMUCK	Gabriele	Bayer Pharma Aktiengesellschaft	DEU
SCHNEIDER	Steffen	BASF SE	DEU
SHAFER	Tim	U.S. Environmental Protection Agency (EPA)	USA
SOGORB	Miguel Angel	Universidad Miguel Hernández de Elche	ESP
SOLECKI	Roland	Federal Institute for Risk Assessment (BfR)	DEU
TERRON	Andrea	European Food Safety Authority (EFSA)	
TSCHUDI-MONNET	Florianne	University of Lausanne	CHE
VALÁŠKOVÁ	Renáta	Research Institute for Organic Syntheses Inc (VUOS)	CZE
VANSTHERTEM	David	Japan Agro Services	BEL
WILKS	Martin	University of Basel	CHE
WITTERS	Hilda	VITO	BEL
ZOELLER	Robert	University of Massachusetts Amherst	USA

ANNEX 4 - ABSTRACTS FOR PRESENTATIONS

Introduction

Why screening for developmental neurotoxicity? – A few thoughts from an epidemiological perspective

Thorhallur Halldorsson, University of Iceland on behalf of European Food Safety Authority (EFSA) [PPT 1]

Background: During the last three decades results from prospective birth cohorts suggest that exposure to chemicals in early life, even at low environmental concentrations, may have long term adverse health consequences. This includes developmental neurotoxicity (DNT), which has received considerable attention following recent reviews of epidemiological findings (Grandjean and Landrigan, Lancet 2006 and Lancet Neurol 2014).

Considerations: Strongest evidence for DNT from epidemiological studies has been reported for inorganic compounds such as lead, mercury and arsenic. That studies have consistently been able to detect adverse associations at levels previously considered safe, may reflect that quantification of inorganic compounds are relatively simple and measures in blood or hair capture past exposures for days or weeks. Evaluating DNT for organic compounds, including pesticides is, however, considerably more complex as these compounds are rapidly metabolized and have short elimination half-life's. As a result, accurate and reliable quantification of exposure is technically difficult. For most organic compounds current evidence for DNT comes from reports on accidental or occupational exposures. In these cases, the exposure is usually far higher than those detected in the general population and extrapolation to lower levels is bound to be uncertain. Even if measured concentrations reflect long term exposures such as for the much studied persistent organochlorine pollutants, reports on DNT from epidemiological studies have been divergent, possibly due to the complexity of aggregating exposure to many compounds that vary in toxicity and pharmacokinetics. Faced with these problems and taking into consideration the cost and time of conducting epidemiological studies, evaluating chemicals in terms of DNT has to be done using multidisciplinary approaches. In vitro screening for DNT for pesticides and other chemicals is a logical first step in that direction.

Conclusion: Based on current knowledge it is hard to justify why mandatory screening for DNT should not be made for chemicals, including pesticides. A tiered approach for regulated products is an option, however capturing the dose response should be part of the assessment as for many identified or suspected compounds DNT appears to be a “simple” question of dose.

Alternative Test Methods for Developmental Neurotoxicity: A History and Path Forward

Kevin Crofton, U.S. Environmental Protection Agency (USA) [PPT 2]

Exposure to environmental contaminants is well documented to adversely impact the development of the nervous system. However, the time, animal and resource intensive EPA and OECD testing guideline methods for developmental neurotoxicity (DNT) are not a viable solution to characterizing potential chemical hazards for the thousands of untested chemicals currently in commerce. Thus, research efforts over the past decade have endeavored to develop cost-effective alternative DNT testing methods. These

efforts have begun to generate data that can inform regulatory decisions. Yet there are major challenges to both the acceptance and use of this data. Major scientific challenges for DNT include development of new methods and models that are “fit for purpose”, development of a decision-use framework, and regulatory acceptance of the methods. It is critical to understand that use of data from these methods will be driven mainly by the regulatory problems being addressed. Some problems may be addressed with limited datasets, while others may require data for large numbers of chemicals, or require the development and use of new biological and computational models. For example mechanistic information derived from in vitro DNT assays can be used to inform weight of evidence (WoE) or integrated approaches to testing and assessment (IATA) approaches for chemical-specific assessments. Alternatively, in vitro data can be used to prioritize (for further testing) the thousands of chemicals used in commerce for which there is no data at all on their potential to cause DNT. The focus of this problem-dictated strategy is that testing is driven by decision-making needs, and the amount of resource utilization is adjusted to provide efficient and timely data to address the needs. As the health and environmental impacts of the decision increase, data needs increase, resource use increases, and the need increases for reduced scientific uncertainty in estimates of risk. Recent advances in testing methods and models hold great promise for the development and use of efficient testing strategies for DNT that are capable of initial prioritization and screening, hazard characterization, and hazard prediction. This abstract does not necessarily reflect U.S. EPA policy.

SESSION 2 / What can be learned from regulatory authorities regarding DNT data requirements and testing strategies based on alternative DNT assays?

EU regulatory perspective with special focus on pesticides

Susanne Hougaard Bennekou, Danish EPA (DNK) and Roland Solecki, German Federal Institute for Risk Assessment (DEU) [PPT 3]

Data requirements for evaluation of plant protection and biocidal products in the EU are legally binding. DNT animal studies or in vitro testing are not mandatory, but may be required for pesticidal active substances, if there is evidence of neurotoxicity on mandatory studies (e.g. neurotoxicity or developmental toxicity studies), to protect the population, including children and woman of childbearing age. Published reviews on the impact of DNT studies for risk assessment of pesticides will be presented. Additionally, 35 available DNT studies out of 485 pesticidal active substances, currently approved in Europe, were reviewed. 19 out of these 35 active substances revealed evidence of DNT. Reference values for only 2 of the 19 positive tested pesticides are based on DNT studies, 2 further substances are currently under discussion. The developmental neurotoxic effects of 15 positive tested substances are covered by both, ADI and ARfD, 2 further positive substances are covered at least by the ADI. In general, the majority of DNT studies did not identify significantly lower NOAELs and LOAELs compared to mandatory studies required for approval of pesticides. Although the majority of risk assessments can be considered protective for positive in vivo DNT effects, for regulatory purposes the identification of potentially hazardous compounds by an adequate in vitro testing battery is considered important as a first screening step.

More and more monitoring data is getting published showing that the vast majority of the population is exposed to pesticides, including children and woman of childbearing age. A recent study in Denmark on exposure to organophosphorus pesticides received large public attention especially in regard to the many untested pesticides and their potential effects on developmental neurotoxicity. This part of the talk will describe the finding of the study, the reaction of the authorities, the scientist, the public and in the end policymakers.

Developmental neurotoxicity under the REACH

Hannele Huuskonen, European Chemicals Agency (ECHA) [PPT 4]

REACH is adopted to improve the protection of human health and the environment from the risks that can be posed by chemicals, while enhancing the competitiveness of the EU chemicals industry. It also promotes alternative methods for the hazard assessment of substances. Information on developmental neurotoxicity (DNT) is required in REACH based on a particular concern on (D)NT as part of an extended one-generation reproductive toxicity study (EOGRTS, OECD TG 443) since 2015. The particular concern for DNT may stem from the information of the substance itself or structurally similar substances. Companies are responsible for the safe uses of their substances and testing proposals should be submitted to ECHA if further information is needed. A testing proposal for an EOGRTS must specify and justify the study design addressing the concerns for the substance in question. ECHA evaluates testing proposals and compliance of dossiers, and issues decisions requesting the adequate studies. Instead of a DNT cohort in an EOGRTS, registrants may propose other studies on DNT in order to clarify the concern. Under substance evaluation further concerns may be addressed. According to Article 13(3) of the REACH Regulation, the test generating information on intrinsic properties of substances shall be conducted in accordance with the test methods laid down in a Commission Regulation or in accordance with other international test methods recognised by the Commission or the Agency as being appropriate. In addition, information on intrinsic properties of substances may be generated in accordance with other tests methods provided that the conditions set out in Annex XI are met. Annex XI describes general rules for adaptation and includes, among others, possibilities such as: use of existing data, weight of evidence and in vitro methods. The data as whole must be adequate for the purpose of classification and labelling including categorisation and risk assessment, although results from an individual adaptation approach may fit only for one of those two. To date non-animal approaches such as IATAs (integrated approaches for testing and assessment) for DNT are rare in the dossiers.

US EPA's Regulatory Perspective on Developmental Neurotoxicity Studies: A Focus on Pesticides

Elissa Reaves, U.S. Environmental Protection Agency (USA) [PPT 5]

The US EPA's Office of Pesticide Programs (OPP) is a licensing program regulating pesticide products in the US. The OPP evaluates the effects of pesticides on human and ecological health. The Food Quality Protection Act of 1996 (FQPA) requires the EPA to add an additional tenfold safety factor to take into account potential pre- and postnatal toxicity and completeness of data with respect to exposure and toxicity to infants and children. This factor may be different only if it is determined that infants and children would be safe. As such, under the FQPA, the OPP may determine the need for more data to inform potential lifestage sensitivity after review of multiple 40 CFR Part 158 guideline studies. The developmental neurotoxicity study (DNT) is a conditionally required study under Part 158 designed to address the potential impact of pesticide exposure on the development of the nervous system. To date, OPP has received and reviewed 101 DNT studies, 18 of which are organophosphates (OPs), and 3 of which are N-methyl carbamates (NMCs). The comparative cholinesterase assay (CCA) is another study design used by the OPP to assess potential lifestage sensitivity. This study evaluates potential differences in cholinesterase inhibition across several lifestages (in utero, perinatal, postnatal, and adulthood) after pesticide exposure. The CCA studies for the OPs and NMCs have proved more sensitive in risk assessment than the DNT. However, of the 101 DNT studies, the OPP has relied upon 24 DNT studies (24%) in setting points of departure for risk assessment. Upon further review of these 24 DNT studies, the offspring were the basis for all 24 DNT LOAELs. Offspring NOAELs were not identified (i.e. the LOAEL was the lowest dose tested) for 7 of the 24 (29%) DNT studies. The offspring effects identified in the 24 DNT studies include: pup mortality in 5 studies, changes in offspring brain morphology in 9 studies, decreased pup weight in 4 studies, behavioral changes in offspring in 5 studies, and developmental delays in 1 study. The need for DNT studies are also being evaluated during the current Registration Review

phase. The OPP has granted 12 waivers for previously required DNT studies and determined that the DNT is required for 2 additional pesticides. In comparison, the OPP has waived the acute neurotoxicity study for 137 of 155 pesticides and the subchronic neurotoxicity study for 149 of 155 pesticides during Registration Review. The OPP is using adverse outcome pathway (AOP) information in its weight of evidence determination for the DNT in risk assessment and if other assays or studies may provide more sensitive data for risk assessment.

US Regulatory Perspective on Developmental Neurotoxicity Testing with Special Focus on Endocrine Disrupting and Industrial Chemicals

Stanley Barone Jr., U.S. Environmental Protection Agency (USA) [PPT 6]

The Endocrine Disruptor Screening Program (EDSP), established under authorities contained in the Federal Food Drug and Cosmetic Act (FFDCA) and the Safe Drinking Water Act (SDWA) amendments required development of a screening program to determine whether certain substances may have endocrine activity in humans and wildlife. One of the key outcomes of developmental endocrine disruption is developmental neurotoxicity. EPA has developed a two tiered approach for screening. EDSP Tier 1 battery is used to identify substances that have potential to interact with estrogen, androgen or thyroid hormone pathways. EDSP Tier 2 assays establish dose response information for adverse effects for substances identified from Tier 1 screening. Currently, neither the EDSP Tier 1 nor Tier 2 battery includes evaluation of neurological function as an endocrine gland or can reveal neurotoxicity as an adverse outcome in the tiered testing paradigm.

EDSP is also advancing the use of ToxCast high throughput screening data and computational models for the purposes of prioritizing and screening a chemical's potential to interact with the endocrine system in humans and wildlife to replace Tier 1 low throughput screening batteries. While this approach will increase the number of chemicals that can be screened for endocrine activity, the current high throughput battery does not cover neurological endpoints in the biological space. The lessons learned from vanguard efforts of the EDSP related to endocrine bioactivity will foster application of these alternative testing methodologies to EDSP and non-EDSP-related evaluation of developmental neurotoxicity and other toxic effects.

The recent passage of Frank R. Lautenberg Chemical Safety for the 21st Century Act which amended the Toxic Substances Control Act only intensifies the requirements and need for prioritization, screening and testing of a large number of chemicals for potential human health environmental risks including developmental neurotoxicity, and other effects related to sensitive subpopulations. To meet this need, OCSPP plans to expand this concept to screen for other endocrine and non-endocrine endpoints.

These new approaches are now being used in a number of decision contexts including EDSP screening and chemical prioritization. Alternative data have also been used in the development of adverse outcome pathway (AOP) frameworks for prediction of adverse outcomes and use in weight of evidence analysis. Significant challenges remain to expand the use of alternative test data, including efforts to address key concerns about developmental neurotoxicity screening and testing.

The views expressed in this abstract do not necessarily reflect US EPA policy.

SESSION 3 / What can be learned from industry's experience with DNT testing strategies based on alternative assays?**EU Industry Perspective: Emphasis on Pesticides**

Gaby Schmuck, representing European Crop Protection Association (BEL) [PPT 7]

Investigation of a pesticide for developmental neurotoxicity (DNT) requires specialized test methods and is of great importance when there is an indication the foetus or neonate is more susceptible than the mother. Therefore, DNT studies are required when the toxicological profile of a compound indicates the potential for neurotoxicity or an indirect mechanism that may affect brain development. This particular risk factor is regulated by EFSA, to determine the risk for operator and consumer in crop use, and by ECHA, as a chemical. The current approach to investigate DNT requires complex neurotoxicity, developmental and reproduction toxicity and DNT studies, with an enormous consumption of animals and limited resources, at great cost. Other toxicological areas have shown the utility of alternatives to in vivo studies (e.g., skin and eye irritation, skin sensitization, genotoxicity and endocrine activity) and in these cases validated methods exist. For DNT, the need for reliable, mechanistic-based assays is welcome, to help guide candidate selection and decision making during development, as well as for registrations. Such assays could offer a better understanding of Mode of Action, which helps determine human relevance and appropriate safety factors to protect infants and children. There are many in vitro models and endpoints available, with various benefits and limitations. The challenge is to select the right complement of models and endpoints for use at the appropriate stages of investigation. To identify and establish models to investigate DNT, we can learn from the experience gained in validating other in vitro models.

U.S. Industry Perspective: DNT Testing Strategies Based on Alternative Assays

Sue Marty, DOW (USA) [PPT 8]

Industry is engaged in both the development and use of alternative models to assess toxicity. These assays offer opportunities to rapidly select candidate molecules for development, provide data on hazard and read across, prioritize chemicals for subsequent screening/testing, and provide information on mode of action. Data from alternative assays, coupled with in vitro-to-in vivo dose extrapolation and exposure data, can aid in distinguishing compounds of low versus high concern. Advancement of alternative assays for developmental neurotoxicity (DNT) screening is challenging as assessing integrated neurodevelopmental function and structure is complex. To evaluate DNT, a battery of assays will be needed to address a diverse array of DNT targets (neurite outgrowth, myelination, etc.), detect a variety of modes of action, and have utility across a broad spectrum of chemistries. These DNT assays should be “fit for purpose” – i.e., greater uncertainty is acceptable for assays used to prioritize compounds for screening, but stringent validation is needed for assays used for regulatory purposes. Comprehensive assay characterization (e.g., positive and negative control compounds, performance criteria, assay confounders/limitations, domain of applicability) will increase assay utility and improve scientific confidence. Lastly, a better understanding of exposure and adverse outcome pathways for DNT (e.g., relationship of key events, magnitude of effect, potency) will improve our ability to use alternative models to predict adverse DNT effects.

SESSION 4 / Why should we encourage the use of DNT testing strategies based on non-animal test methods?

Perspectives on how the Adverse Outcome Pathway concept informs the use of in vitro DNT data for regulatory purposes

Anna Price, European Commission Joint Research Centre (JRC) [PPT 9]

The AOP concept relies on understanding causal relationship between the Molecular Initiating Event (MIE), in which a chemical interacts with a biological target, resulting in a sequential series of measurable key events (KEs), which are triggered at different biological levels (cellular, tissue, organ) ultimately resulting in adverse outcome (AO) manifesting in an individual organisms and/or a population. DNT AOPs hold great potential to impact the manner in which in vitro DNT data can be interpreted since the causative links between MIEs, KEs and AO are based on empirical, mechanistic data and biologically relevant knowledge, providing more certainty for regulatory use.

Moreover, AOPs provide a strong biological/pathophysiological rationale to compound classification, which is usually based on chemical structures correlated to apical endpoints from animal experiments. It is an important tool that facilitates generation of the data needed for formation of chemical biological categories: chemicals can be grouped according to their MIEs, and common KEs. AOP-based biological chemical grouping has the potential to add a value for DNT testing due to the complex nature of the underlying biology that is currently inadequately captured by chemical category formation (structure or reactivity). Furthermore, read-across and toxicity classification models can be vastly improved when large amounts of in vitro data are available from high-throughput testing. However, currently the limited number of the developed DNT AOPs has hampered both judgement of the predictive ability, as well as regulatory use of high-throughput in vitro DNT data.

The concept that underlies the AOP framework can also guide more effective selection of existing in vitro DNT data and can advise on the most relevant in vitro tests to be included in Integrated Approaches to Testing and Assessment (IATA) for generation of new data reflecting appropriate coverage of MIEs and KEs.

In this presentation possible AOP applications in regulatory context will be discussed based on examples of the existing DNT AOPs.

How to link test systems to the prediction of developmental neurotoxicity (DNT)

Marcel Leist, University of Konstanz (DEU) [PPT 10]

As DNT is assumed to result from the modulation of fundamental neurodevelopmental processes (such as neuronal differentiation, precursor cell migration or neuronal network formation) by chemicals, the first generation of alternative DNT tests target these processes. The advantage of such types of assays is that they capture toxicants with multiple targets and modes-of-action. Moreover, the processes modelled by the assays can be linked to toxicity endophenotypes (TEP), i.e. alterations in neural connectivity that form the basis for neurofunctional deficits in man. The concept of ‘toxicity endophenotypes’ focuses on fundamental biological processes of relevance to adverse outcomes at the organismal level that can be modelled by in vitro systems. Characteristic adverse outcomes in the field of DNT are cognitive or psychomotor deficits, including reduced IQ, attention deficit, ataxia or various sensory disturbances, in addition to malformations (e.g. spina bifida or microcephaly). They describe external/apical phenotypes that are functionally defined, and which are difficult to model using presently-known in vitro systems. Most knowledge on human DNT compounds relates to these externally manifested functional phenotypes (= exophenotypes). For development of relevant model systems, we need approaches to link the ‘exophenotype’, caused by xenobiotic exposure in the intact organism, to the effects the compound triggers

in in vitro test systems. TEP describe the altered functional or structural connectivity or responsiveness of parts of the nervous system triggered by xenobiotics. All developmental neurotoxicants are expected to affect at least one fundamental biological process in vivo, and this would result in an altered TEP. Thus, TEP represent a key link between the known effects of DNT chemicals and their effects in in vitro systems.

SESSION 5 / Discussion Group (DG) sessions: OECD case studies for potential testing strategies based on non-animal test methods and a draft framework for building a DNT testing battery

Introduction to OECD case studies for potential testing strategies and a draft framework for building a DNT testing battery

Ellen Fritsche, Dusseldorf University (DEU) [PPT 11]

During brain development neurodevelopmental processes are guided by distinct signalling pathways and orchestrated over time. This knowledge is utilized for novel approaches for developmental neurotoxicity (DNT) testing: dissection of complex brain formation into individual neurodevelopmental processes that can be studied in a dish in vitro. The idea is that interference with of any of the processes necessary for proper brain development will lead to an adverse outcome. Because individual processes are guided by specific signalling pathways, it is implied that a battery of individual test needs implementation into the alternative testing strategy; it is not sufficient to study just one of the neurodevelopmental processes. Such an in vitro testing strategy is proposed to be comprised of 5 to 6 assays covering a multitude of neurodevelopmental processes. Some of these will be presented by using a case study: neurodevelopmental effects of methylmercury. As an in vitro test is not a stand-alone method, it is furthermore proposed that the in vitro test battery might be part of an integrated testing strategy. Here, the first tier would be kinetic modelling of the compound to understand the order of magnitude of internal exposure in the human developing brain in vivo. The next tier would contain the in vitro testing battery followed by alternative model organism testing, e.g. in the developing zebrafish. In case there is concern that would call for targeted in vivo testing in the rat, another tier, rodent in vitro testing, would be infixed that ensures a species-independent mode-of-action of the compound. This is necessary because in a targeted in vivo approach investigations are based on the compound's mode-of-action.

In summary, such an alternative, tiered DNT testing approach is faster and cheaper than current in vivo testing, takes mode-of-action and species effects into consideration, and is thus thought to be an improvement of the current DNT testing paradigm.

ANNEX 5 – SLIDES OF SPEAKERS' PLENARY PRESENTATIONS

Please refer to the separate publication for full Annex 5

ENV/JM/MONO(2017)87/ANN2

[PPT 1] Why screening for developmental neurotoxicity? – A few thoughts from an epidemiological perspective

Thorhallur Halldorsson, University of Iceland on behalf of European Food Safety Authority (EFSA)

[PPT 2] Alternative Test Methods for Developmental Neurotoxicity: A History and Path Forward

Kevin Crofton, U.S. Environmental Protection Agency (USA)

[PPT 3] EU regulatory perspective with special focus on pesticides

Susanne Hougaard Bennekou, Danish EPA (DNK) and Roland Solecki, German Federal Institute for Risk Assessment (DEU)

[PPT 4] Developmental neurotoxicity under the REACH

Hannele Huuskonen, European Chemicals Agency (ECHA)

[PPT 5] US EPA's Regulatory Perspective on Developmental Neurotoxicity Studies: A Focus on Pesticides

Elissa Reaves, U.S. Environmental Protection Agency (USA)

[PPT 6] US Regulatory Perspective on Developmental Neurotoxicity Testing with Special Focus on Endocrine Disrupting and Industrial Chemicals

Stanley Barone Jr., U.S. Environmental Protection Agency (USA)

[PPT 7] EU Industry Perspective: Emphasis on Pesticides

Gaby Schmuck, representing European Crop Protection Association (BEL)

[PPT 8] U.S. Industry Perspective: DNT Testing Strategies Based on Alternative Assays

Sue Marty, DOW (USA)

[PPT 9] Perspectives on how the Adverse Outcome Pathway concept informs the use of in vitro DNT data for regulatory purposes

Anna Price, European Commission Joint Research Centre (JRC)

[PPT 10] How to link test systems to the prediction of developmental neurotoxicity (DNT)

Marcel Leist, University of Konstanz (DEU)

[PPT 11] Introduction to OECD case studies for potential testing strategies and a draft framework for building a DNT testing battery

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