

Alternative Tests und Teststrategien für REACH und die EU-Kosmetik-Richtlinie

Richard Vogel

Zentralstelle zur
Erfassung und
Bewertung von
Ersatz- und Ergänzungsmethoden zum
Tierversuch

Bundesinstitut
für
Risikobewertung
*Federal Institute
for Risk Assessment*

ZEBET - Zentralstelle zur Erfassung und Bewertung von Ersatz- und Ergänzungsmethoden zum Tierversuch



Risiken erkennen – Gesundheit schützen

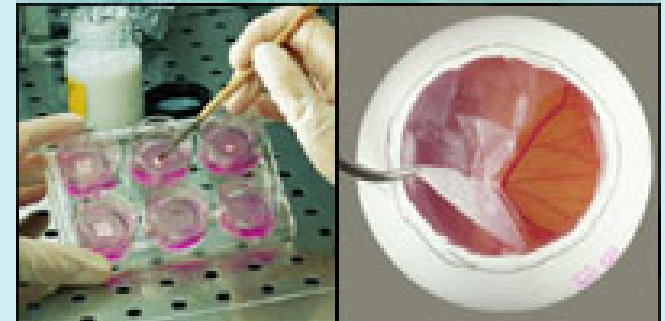
Aufgaben und Ziele

Umsetzung des 3R Konzeptes zum Ersatz von Tierversuchen: Refine – Reduce – Replace

- Entwicklung und Validierung von Alternativmethoden
- Implementierung neuer Methoden in Richtlinien
- Training und Kurse zu Alternativmethoden
- Dokumentation von Alternativmethoden (Datenbank AnimAlt ZEBET)
- Forum zum Informationsaustausch
- Beratung und Information aller Interessengruppen

Forschungsschwerpunkte

- Reproduktionstoxikologie
- Inhalationstoxikologie
- Lokale Toxizität an Haut und Auge
- Informationsbeschaffung zu Alternativmethoden über das Internet



5. Anwendungsreife Etablierung einer Richtlinie (z.B. OECD)	10 Jahre	Behörden
4. Validierung ESAC-Statement Peer-Review Relevanz und Zuverlässigkeit für Anwendungsbereich Ringversuch	3 Jahre	BMBF / EU
3. Prä-Validierung Inter-Labor Vergleich Verbesserung des Testprotokolls Verbesserung des Prädiktionsmodells	2 Jahre	BMBF / EU
2. Testentwicklung Prädiktionsmodell Testprotokoll	5 Jahre	BMBF / EU
1. Grundlagenforschung wissenschaftliche Basis Anwendungsbereich	3 Jahre	ZEBET / DFG

Testanforderungen unter REACH für die Säugertoxikologie

- Akute Toxizität (oral, dermal, inhalativ)
- Augenreizung/-ätzung
- Hautreizung/-ätzung
- Sensibilisierung der Haut

- **Mutagenität / Karzinogenität**

- (sub-) chronische Toxizität
- Reproduktionstoxizität
 - Fertilität und Entwicklungstoxizität
- Karzinogenität, nicht mutagen

Wissenschaftlich validierte Methoden

Akute Toxizität

Acute Toxic Class Method for Acute Oral Toxicity Testing

Date of the ESAC statement: 31 October 2007

Links: [ESAC Statement](#)

Fixed Dose Procedure for Acute Oral Toxicity Testing

Date of the ESAC statement: 31 October 2007

Links: [ESAC Statement](#)

Up-and-Down Procedure for Acute Oral Toxicity Testing

Date of the ESAC statement: 31 October 2007

Links: [ESAC Statement](#)

Wissenschaftlich validierte Methoden

Hautätzung (und Hautreizung)

CORROSITEX assay for skin corrosivity

Date of the ESAC statement: 06 December 2000

Links: [INVITTOX Protocol](#), [ESAC Statement](#)

EpiSkin™ skin corrosivity test

Date of the ESAC statement: 03 April 1998

Links: [INVITTOX Protocol](#), [ESAC Statement](#)

Rat Transcutaneous Electrical Resistance (TER) skin corrosivity test

Date of the ESAC statement: 03 April 1998

Links: [INVITTOX Protocol](#), [ESAC Statement](#)

EpiDerm™ skin corrosivity test

Date of the ESAC statement: 21 March 1998

Links: [INVITTOX Protocol](#), [ESAC Statement](#)

Wissenschaftlich validierte Methoden

Augenätzung (und Augenreizung)

The Bovine Corneal Opacity and Permeability (BCOP) and the Isolated Chicken Eye (ICE) test methods for eye irritation

Date of the ESAC statement: 27 April 2007

Links: [ESAC Statement](#)

Wissenschaftlich validierte Methoden

Sensibilisierung

Reduced Local Lymph Node Assay (rLLNA) for skin sensitisation

Date of the ESAC statement: 27 April 2007.

Links: [ESAC Statement](#)

Local Lymph Node Assay for skin sensitisation (LLNA)

Date of the ESAC statement: 21 March 1999

Links: [ESAC Statement](#)

Wissenschaftlich validierte Methoden

Entwicklungstoxizität

Embryonic stem cell test for embryotoxicity

Date of the ESAC statement: 01 May 2002

Links: [INVITTOX Protocol](#), [ESAC Statement](#)

Micromass embryotoxicity assay

Date of the ESAC statement: 01 May 2002

Links: [INVITTOX Protocol](#), [ESAC Statement](#)

Whole rat embryo embryotoxicity assay

Date of the ESAC statement: 01 May 2002

Links: [INVITTOX Protocol](#), [ESAC Statement](#)

Welche der validierten Methoden sind OECD-Guidelines ?

Regulatorische Akzeptanz

Akute Toxizität

Fixed Dose Procedure for Acute Oral Toxicity Testing

Regulation: OECD Test Guideline 420 adopted December 2001

Links: [67/548/EEC Annex V](#), [OECD](#)

Acute Toxic Class Method for Acute Oral Toxicity Testing

Regulation: OECD Test Guideline 423 adopted December 2001

Links: [67/548/EEC Annex V](#), [OECD](#)

Up-and-Down Procedure for Acute Oral Toxicity Testing

Regulation: OECD Test Guideline 425 adopted December 2001

Links: [OECD](#)

=====

Deletion of the acute oral toxicity test, Lethal Dose (LD50)

Regulation: the method has been deleted in 2001 from both the Annex V of Council Directive 67/548/EEC (Method B.1.), as well as from the OECD Test Guidelines (TG 401).

Links: [67/548/EEC Annex V](#), [OECD](#)

Animal Numbers and Costs in Acute Toxicity Testing

Route	Test	Rats	Euro
=====			
Oral	OECD 401 LD50	40	2.000
	OECD 423 ATC	9	1.900
	Halle Register	3	
		0	300
=====			
Dermal	OECD 402 LD50	40	2.900
	OECD ??? ATC	(9)	
=====			
Inhalation	OECD 403 LC50	40	20.500
	OECD ??? Cultex	(0)	6.000

Regulatorische Akzeptanz

Hautätzung

EpiSkin™ skin corrosivity test

Regulation: included into Annex V of Council Directive 67/548/EEC part B.40 on skin corrosion in April 2000; and Draft OECD Test Guideline 431 approved in May 2002.

Links: [67/548/EEC](#), [OECD](#)

EpiDerm™ skin corrosivity test

Regulation: included into Annex V of Council Directive 67/548/EEC part B.40 on skin corrosion in April 2000; and Draft OECD Test Guideline 431 approved in May 2002.

Links: [67/548/EEC](#), [OECD](#)

Rat TER skin corrosivity test

Regulation: included into Annex V of Council Directive 67/548/EEC part B.40 on skin corrosion in April 2000; and Draft OECD Test Guideline 430 approved in May 2002.

Links: [67/548/EEC](#), [OECD](#)

Animal Numbers and Costs in Irritation/Corrosivity Testing

Route	Test	Rabbits	Euro
=====			
Skin	OECD 404	3	1.300
	OECD 431 EpiDerm	0	1.300
=====			
Eye	OECD 405	3	1.400
	OECD ??? BCOP/ICE	0	?
	OECD ??? HET-CAM	0	1.400

Regulatorische Akzeptanz

Sensibilisierung

Local Lymph Node Assay for skin sensitisation (LLNA)

Regulation: Updated OECD Test Guideline 429, adopted 24 April 2002; and U.S. EPA - OPPTS Harmonized Test Guideline 870.2600 on Skin Sensitization, August 1998

Links: [OECD](#), [U.S. EPA OPPTS](#)

Animal Numbers and Costs in Sensitivity Testing

Route	Test	Animals	Euro
===== Skin	OECD 406 Bühler	30 g-pigs	6.200
	OECD 429 LLNA	16 mice	3.800

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Technical Guidance Document to Industry on the Information Requirements for REACH

EWG 1-7

**REACH Implementation Project
(RIP) 3.3 Phase 2**

1.2 INFORMATION REQUIREMENTS

The standard data requirements for reproductive toxicity under the REACH Regulations are as follows:

- A prenatal developmental toxicity study (OECD TG 414) in one species, usually required at the REACH Annex IX level. A study in a second species should be considered at either Annex IX or at Annex X level.
- A two-generation reproduction toxicity study^[1] (OECD TG 416) in one species, usually required at the Annex X level.

^[1] A proposed 'F1-extended one-generation study', as discussed in 1.4.1, may replace OECD TG 416 as a definitive study for reproductive toxicity in the near future, subject to gaining regulatory acceptance in the EU

Animal Numbers and Costs in Reproductive Toxicity Testing

Endpoint	Test	Animals	Euro
=====			
Dev. Tox.	OECD 414 rat	150	76.000
	OECD 414 rabbit	150	110.000
=====			
Fertility	OECD 416 rat	2.600	345.000

Problem: Reproductive Toxicity Testing

Dominant test demand: 70 - 80% of animal use in REACH for 3.000 substances

Lacking capacities and experience (e.g. only 70 two-gen-studies in 25 years for industrial chemicals)

**Need for new tests and
for new test strategies**

Alternative Methods for Reprotox ?

Developmental Toxicity

Murine embryonic stem cell test (mEST)

Date of the ESAC statement: 01. May 2002

OECD TG ???

Human embryonic stem cell test (hEST)

Date of the ESAC statement: ???

OECD TG ???

Fertility Impairment

(Extended) One Generation Reproduction Toxicity Study

Date of the ESAC statement: ???

OECD Test Guideline 415

2-Generation Study (OECD TG 416)

F0 parental animals

Males $4 \times 25 = 100$

Females $4 \times 25 = 100$

F1 offspring $80 \times 15 = 1200$

F1 parental animals from F1 offspring

Males 0

Females 0

F2 offspring $80 \times 15 = 1200$

=====

OECD 416: 2600 animals

1-Generation Study (OECD TG 415)

F0 parental animals

Males $4 \times 25 = 100$

Females $4 \times 25 = 100$

F1 offspring $80 \times 15 = 1200$

=====

OECD 415: 1400 animals

Reduction: 1200 animals per study

OECD GUIDELINES FOR TESTING OF CHEMICALS

416

Two Generation Study

adopted 26 May 1983
revised 22 January 2001

415

One Generation Study

adopted 26 May 1983

OECD TEST GUIDELINES PROGRAMME
Standard Project Submission Form

PROJECT TITLE:

Update of the 1-Generation Study OECD 415

SUBMITTED BY:

Richard Vogel

DATE OF SUBMISSION TO THE SECRETARIAT:

January 20, 2007

**Potential Reduction in Animal Usage****Current testing guidelines**

• 2 species Dev. Tox (<i>parental</i>)	160
• 2 Gen Reprotox (<i>parental & offspring</i>)	2600
• Dev. Neurotox (<i>parental & offspring</i>)	1280
• Dev. Immunotox (<i>parental & offspring</i>)	<u>1280</u>
	5320

Tier I testing only

• 1 species Dev. Tox (<i>parental</i>)	80
• Extended 1-Gen Reprotox (<i>parental & offspring</i>)	<u>1400</u>
	1480

OECD TEST GUIDELINES PROGRAMME
Standard Project Submission Form

PROJECT TITLE

(Extended) One Generation Study

SUBMITTED BY (Country / European Commission / Secretariat)

United States

Germany

The Netherlands

DATE OF SUBMISSION TO THE SECRETARIAT

2007-05

Step 1 would replace the current one-generation study Test Guideline (OECD 415). It is believed that the revised one-generation Test Guideline could be used for most applications in chemicals assessment in lieu of the mammalian 2-generation study (OECD 416).

Step 2 (combined with step 1) could replace the current developmental neurotoxicity study (OECD 426) and are expected to provide more endocrine sensitive endpoints in combination with neurological and immunological endpoints as well as enhance sensitivity by retaining more pups/litter for necropsy as adults than current TGs. This Guidance Document could be converted to a Test Guideline after more experience is gained in its use.

Extended One-Generation Reproductive Toxicity Study

Table of Contents:

Introduction

Initial considerations and objectives

Principle of the test

Description of the method / Preparations for the test

Animals

Test substance

Procedures

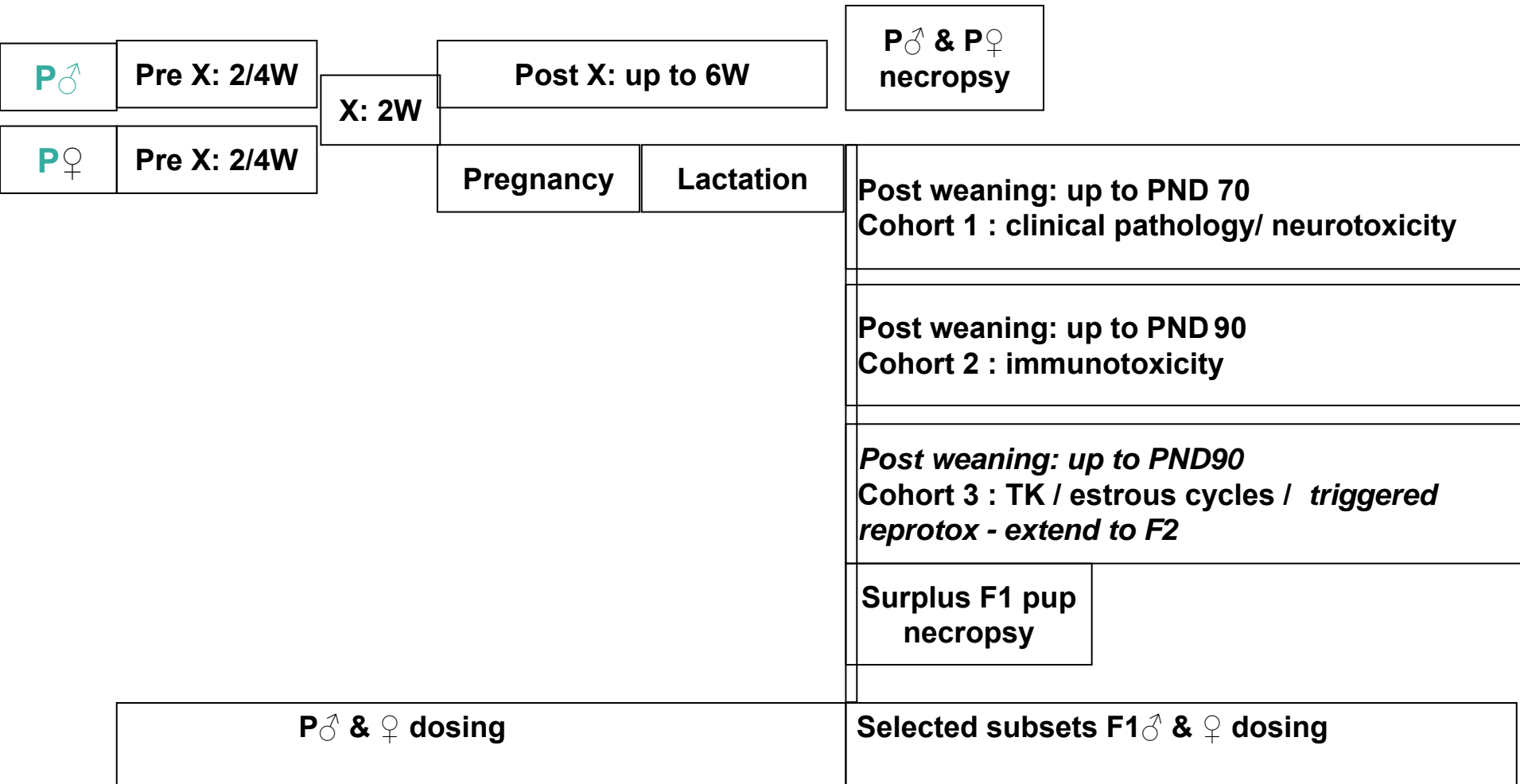
In life observations

Terminal observations

Reporting

Interpretation of results

Extended One-Generation Protocol



Modular One-Generation Reproductive Toxicity Study

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Principle of the test

I. The Core One-Generation Reproduction Toxicity Study

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II. Triggered Modules

Options for extension modules

Procedures

Module 1 “second mating or second generation”

Module 2 “developmental toxicity”

Module 3 “developmental neurotoxicity”

Dosing of P males and females

Dosing of F1
Necropsy P and surplus F1

Premating
4 weeks

Mating
4 weeks

Pregnancy

F1 pups
Lactation

F1 pups
up to
PND 90

TRIGGER

2. mating or
2. generation
(OECD 416)

Developmental
Toxicity
(OECD 414)

Developmental
Neurotox
(OECD 426)

Animal Numbers and Costs for Generation Studies

Endpoint	Test	Animals	Euro
Fertility	OECD 416	2.600	345.000
	OECD 415	1.400	185.000
	OECD Extended	1.400*	600.000

*größere Belastungen der F1

Editorial

REACH Testing Requirements Must Not be Driven by Reproductive Toxicity Testing in Animals

Horst Spielmann and Richard Vogel⁴

2-generation study must not be mandatory at all !

An updated 415 should be standard requirement under REACH

An updated 415 should be extended to cover additional endpoints in the scope of pesticide assessment.

Danke für Ihre Aufmerksamkeit !

Richard Vogel

ZEBET

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Förderung von Alternativmethoden in der EU FP6 & FP7

Funding scheme	Number of projects	EC financial contribution (million €)
Sixth FP		
Integrated project	4	39.5
Specific Targeted Research Project	4	8.7
SME-Specific Targeted Research Project	5	13.0
Specific Support Action	8	2.1
Total	21	63.3
Seventh FP (First Call)	5	30.4 (planned)

Förderung von Alternativmethoden in Deutschland *pro Jahr*



**4,0 Mio €
(seit 1980)**



**0,35 Mio €
(seit 1990)**

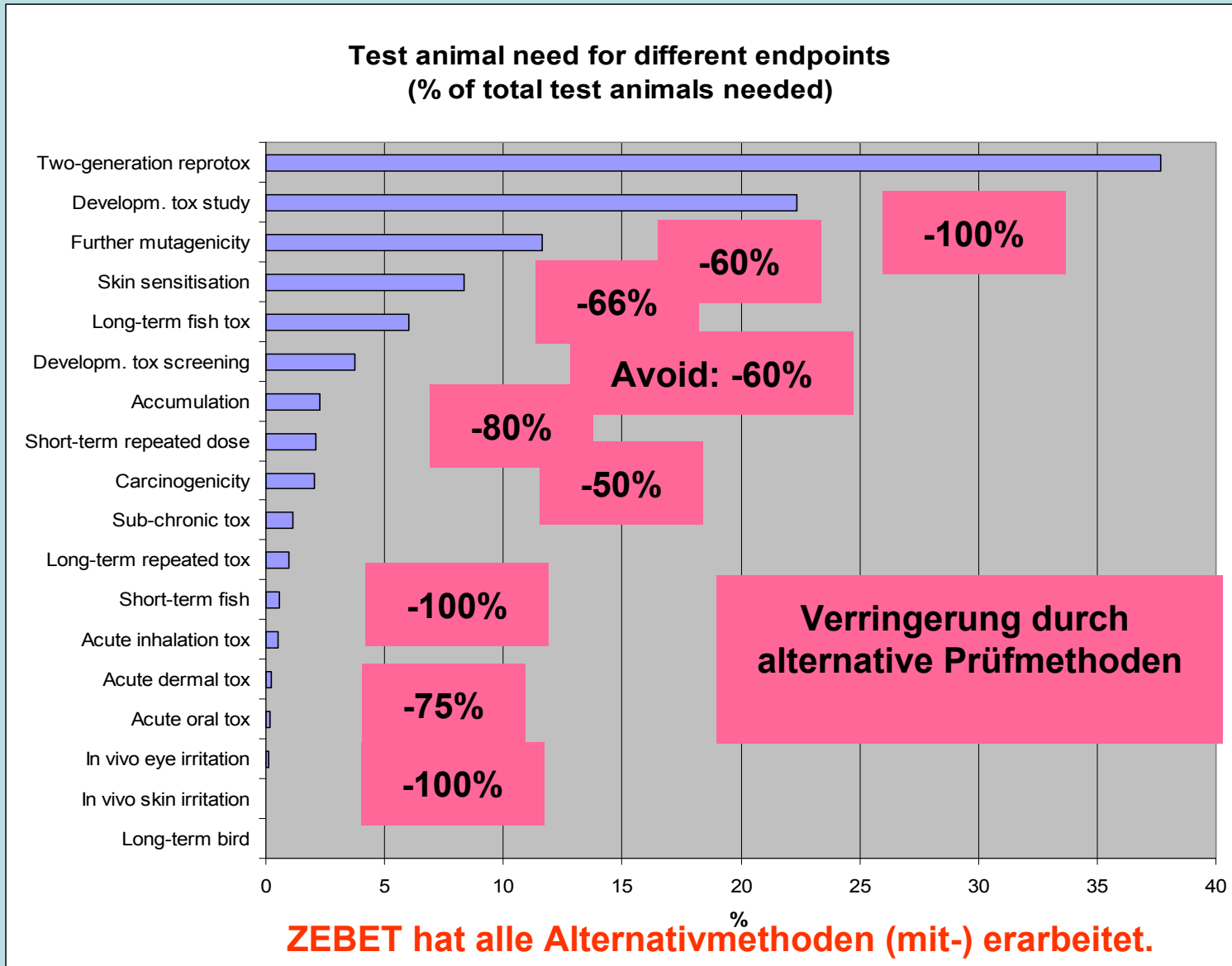


**0,2 Mio €
(seit 1987)**

**Baden-Württemberg,
Rheinland-Pfalz**

EU Chemikalienrichtlinie REACH:

Tierverbrauch für die einzelnen Prüfmethode(n) (van der Jagt et al., 2004)



-50%

**Verringerung durch
alternative Prüfmethode(n)**

Issues to be discussed:

1. Loss of information without second generation ?
2. Validation ?
3. Triggers for extensions ?