German Environment Agency

Umwelt Bundesamt

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„Bioassays to assess unpredictable substances from drinking water contact materials "

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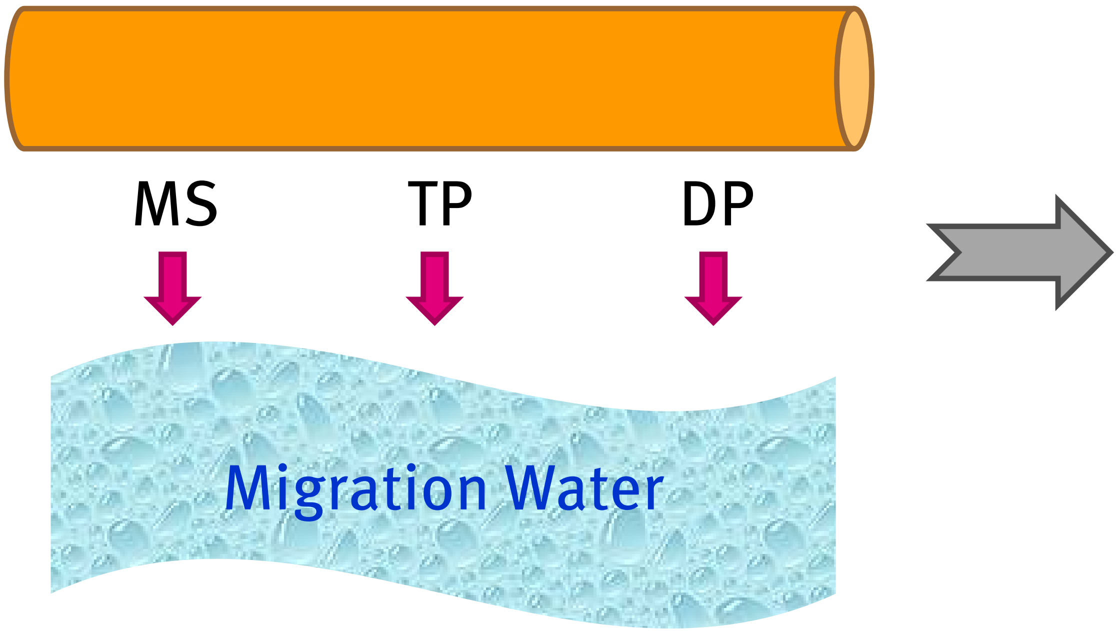
Project:
Bioassay-based test strategy to determine risk potentials of migration waters

GERMAN ENVIRONMENT AGENCY (UBA)

+

THE ASSOCIATION FOR PIPE SYSTEMS INSIDE BUILDINGS (VRH)

Starting point



Identification of toxicological risk potentials

MS: migration of starting substances
TP: transformation products
DP: degradation products

Current Assessment

- GATHERING AND ASSESSMENT OF STARTING SUBSTANCES AS WELL AS POSSIBLE REACTION PRODUCTS FROM THE PRODUCTION OF MATERIALS IN CONTACT WITH DRINKING WATER
- CREATION OF MIGRATION WATERS ACCORDING TO THE GERMAN STANDARD DIN 12873-1
- ANALYZIS OF THE MIGRATION WATER BY SINGLE SUBSTANCE CHEMICAL ANALYSIS FOR SUBSTANCE MIGRATION FROM THE MATERIAL
- SETTING OF MAXIMAL ALLOWED SUBSTANCE MIGRATIONS FROM THE MATERIAL IN CONTACT WITH DRINKING WATER ACCORDING TO TOXICOLOGICAL ASSESSMENT FOR THESE SINGLE COMPOUNDS AND IN ACCORDANCE WITH THE PRESENT KTW GUIDELINE AND THE FUTURE UBA EVALUATION CRITERIA DOCUMENT FOR ORGANIC MATERIALS

THIS WILL BE CONTINUED!

Limits of current assessment

- A HIGH NUMBER OF STARTING COMPOUNDS AND REACTIONS PRODUCTS IN CASE OF CERTAIN MATERIALS
- MANY MIGRATING SUBSTANCES AT VERY LOW CONCENTRATIONS
- OCCURRENCE OF UNEXPECTED REACTION AND DEGRADATION PRODUCTS
- COMPLEX SUBSTANCE MIXTURES

IN THESE CASES A COMPREHENSIVE ASSESSMENT IS HARDLY POSSIBLE!

Solution: Results of Tox-Box-Project

→ Harmonized test strategy

→ User-related criteria

Test program

- 6 test series / migration waters of different materials according to DIN 12873-1
- Tox-Box program
  - Genotoxicity
  - Neurotoxicity
  - Endocrine effects
- Definition of a test and assessment strategy

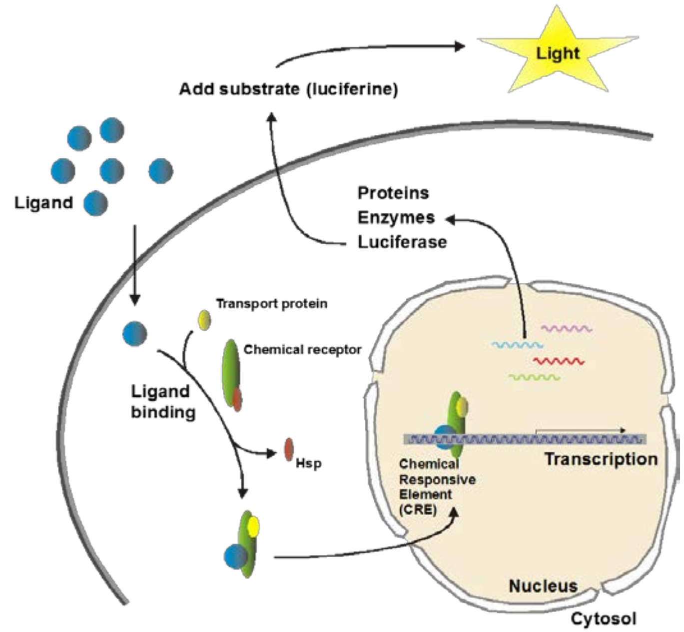
Test strategy genotoxicity

In Vitro Tests

Bacterial test	*Cytotoxicity*	Cell culture
Ames I	ROS	Micronucleus test

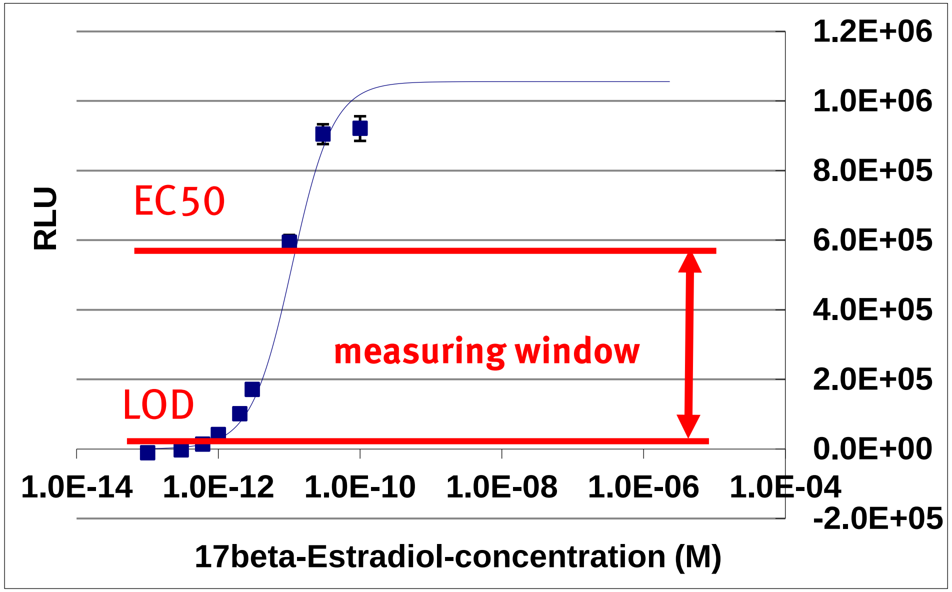
Test strategy endocrine effects

Estrogene-like activity (ERα-CALUX)
migration waters + extracts



Biodetection Systems, product information 2008

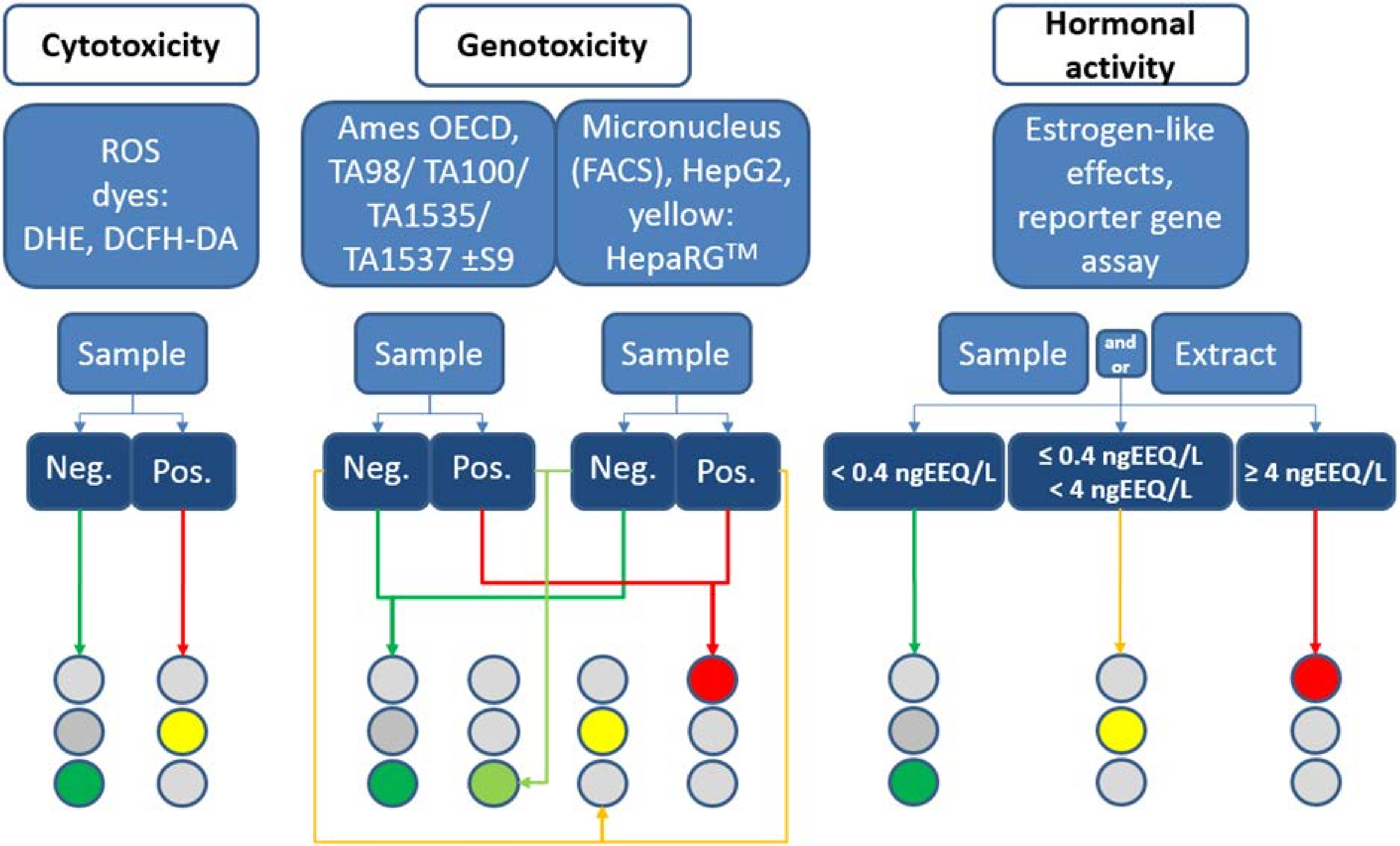
Dose response curve for effect quantification



Assessment criteria

Color coded ranking	Result	Assessment
	Positive in one endpoint specific test strategy (combination of 2-3 in vitro tests) for genotoxicity, neurotoxicity or endocrine activity	Endpoint specific toxicological risk potential
	Positive in single in vitro tests	Suspicion for toxicological risk potential
	No positive results	No toxicological risk potential

Assessment criteria



Source: German Environment Agency

DHE positive › 10% fluorescence
DCFH-DA positive › 20 nM DCF

AMES: positive IF ≥1,2 / micronucleus positiv IF ≥ 2

Results I

Test conditions: 24 h / 85°C

Material	Sample	S/V dm(-1)	TOC mg/dm2 x d	TOC mg/l	Toxicological assessment: genotox.			Estrogenic Activity			cytotox. ROS		Σ	comp. with current DW approval
Pipe 1	1	33			x			x			x			
	2	33			x			x			x			
Pipe 2	1	33			x			x			x			
	2	33			x			x			x			
Pipe 3.1	1	27			x			x			x			
	2	27	1.11	29.97	x				x		x			
Pipe 3.2	1	27	9.28	246.85	x			x			x			
Pipe 4	1	25			x			x			x			
	2	25	0.08	2.01	x			x			x			
Pipe 5	1				x			x			x			
	2				x			x			x			
Pipe 6	1	29	0.06	1.83	x			x			x			
Pipe 7	1	28	12.95	360	x			x			x			
	2	28	5.25	146	x			x			x			
Pipe 8	1	28	0.03	0.77	x			x			x			
	2	28	0.03	0.79	x			x			x			
Pipe 9	1						x		x			x		

Summary I

Toxicological risk potentials detectable,
but:

- No genotoxic effects from materials with current drinking water (DW) approval
- Pipe materials with current DW approval showed no estrogene- like effects at 85°C
- Neurotoxicity no assessment relevant endpoint with current available test methods (possible follow-up project)

Results II
Reproducibility of estrogene-like activity at the lower detection range

	Migration tests with conditions				Assessment = estrogene-like activity of extracts			Mean Extracts (ngEEQ/L)	Overall Assessment
Material	No of parallels	S/V (dm-1)	T (°C)	MigrSt x t(h)	green	yellow	red		
Positive Control	1	37	60	1 x 24			x	32.9	
	1			3 x 24			x	76.9	
	1			7 x 24			x	65.9	
	1		23	1 x 72			x	29.6	
	1			3 x 72			x	25.6	
Pipe with DW-certificate	1	29	60	1 x 24	x			< LOD	
	1			3 x 24	x			< LOD	
	1			7 x 24	x			< LOD	
	1		23	1 x 72	x			< LOD	
	1			3 x 72	x			< LOD	
Elastomer 1.1 undervulcanized	1	5	60	1 x 24		x		0.6	
	1			3 x 24		x		0.5	
	1			7 x 24		x		0.8	
	1		23	1 x 72		x		0.6	
	1			3 x 72		x		1.1	
Elastomer 1.2 undervulcanized new sample	**5**	5	60	0 x 24		x (5x)		0.68	
	5			7 x 24	x (1x)	x (4x)		0.63	
	5			24 x 24	x (4x)	x (1x)		0.38	
	5		23	0 x 24	x (5x)			0.15	
	5			3 x 72	x (1x)	x (4x)		0.54	
	5			9 x 72	x (5x)			0.34	

Results II

	Migration tests with conditions					Assessment = estrogene-like activity of extracts			Mean Extracte (ngEEQ/L)	Overall Assessment
Material	No of parallels	S/V (dm-1)	T (°C)	MigrSt x t(h)		green	yellow	red		
Elastomer 3.2 optimal vulc	1	5	60	1 x 24		x			< LOD	
	1			3 x 24		x			< LOD	
	1			7 x 24		x			< LOD	
Elastomer 4 optimal vulc	1	50	60	1 x 24			x		0.8	
	1			3 x 24			x		0.6	
	1			7 x 24			x		0.6	
	4	5	60	0 x 24		x (1x)	x (3x)		0.42	
	5			7 x 24		x (4x)	x (1x)		0.37	
	5			24 x 24		x (5x)			0.14	
	5		23	0 x 24		x (4x)	x (1x)		0.18	
	5			3 x 72		x (5x)			0.27	
	5			9 x 72		x (5x)			0.11	

Summary II

- Positive control material shows very high estrogene-like activity
- Estrogene-like activity are time dependently reduced in case of materials with current drinking water approval
- Clear differences between materials and in some cases between producers variant measurable
- Degree of vulcanization determines type and level of estrogene-like effects: estrogene-like effects of poor cross-linked mixtures may vanish after optimized vulcanization

UBA-Publication

German Enviroment Agency

28. February 2018

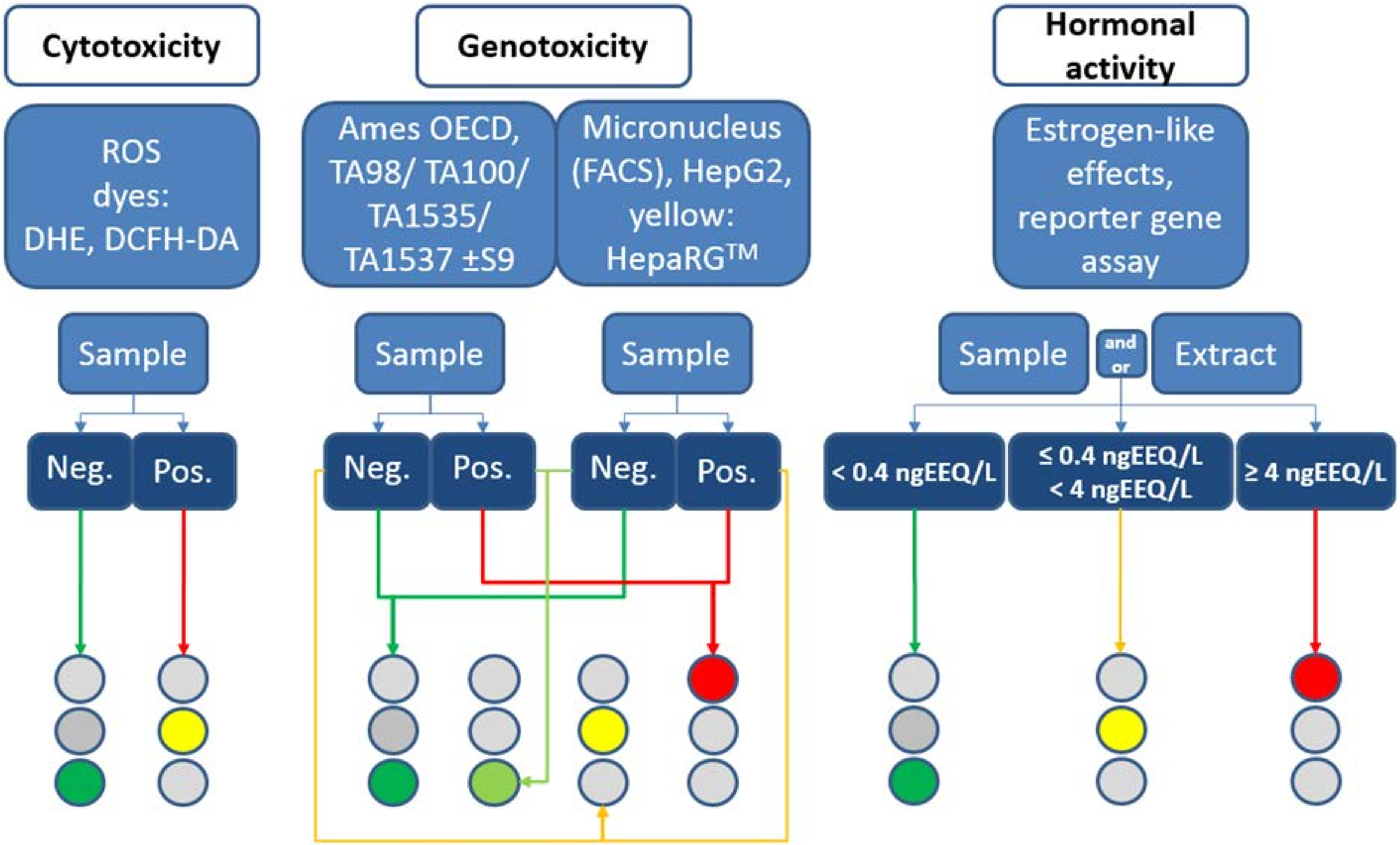
Bioassays to assess materials in contact with drinking water

Assessment of unpredictable substances

Within the project „bioassay-based test strategy to determine risk potentials of migration waters“ the German Environment Agency (UBA) and the Association for Pipe Systems Inside Buildings (VRH) had the common aim to develop a test strategy, that is based on well-established bioassays. This *in vitro* test strategy detects cytotoxicity, genotoxicity and estrogenic activity of migration waters. These effects are not only unwanted in migration water, but in all products. Especially since these effects pose a risk to human health and the environment they are relevant for assessment. In some cases migration water of organic materials in contact with drinking water contains reaction and degradation products, as well as contaminants of the starting substances, which are currently not assessed. This new approach also accounts for these contaminants.

https://www.umweltbundesamt.de/publikationen/bioassays-to-assess-materials-in-contact-drinking

Assessment criteria



Source: German Environment Agency

Conclusion

- Tox-Box strategy applicable for migration waters
- Development work necessary for endocrine effects
- Assessment of substance mixtures (total load of TP, RP, DP) possible
- Identification of effect causing substance(s) should be aimed for

Thank You for Your Attention

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