

ENVIRONMENTAL SCIENCE FOR THE EUROPEAN REFINING INDUSTRY

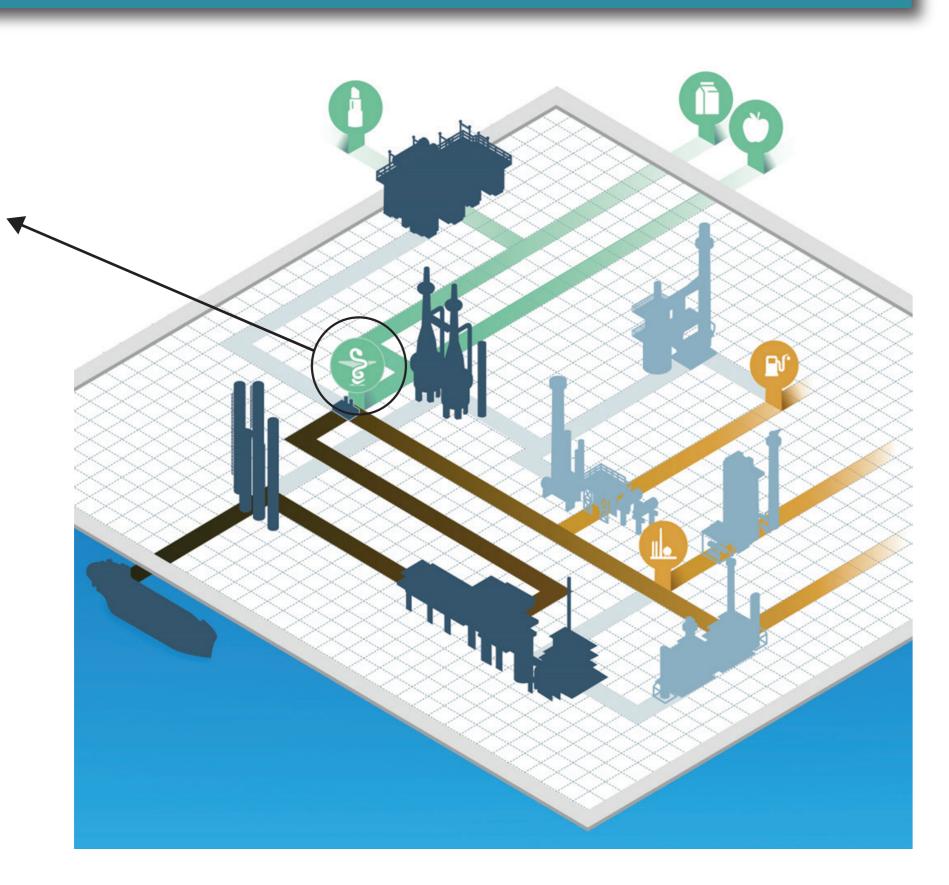
RATIONALE

"Gatekeeper":

IP346 and Modified Ames test are fundamental and rapid screening assays for carcinogenicity and mutagenicity of petroleum streams (EC 1272/2008).

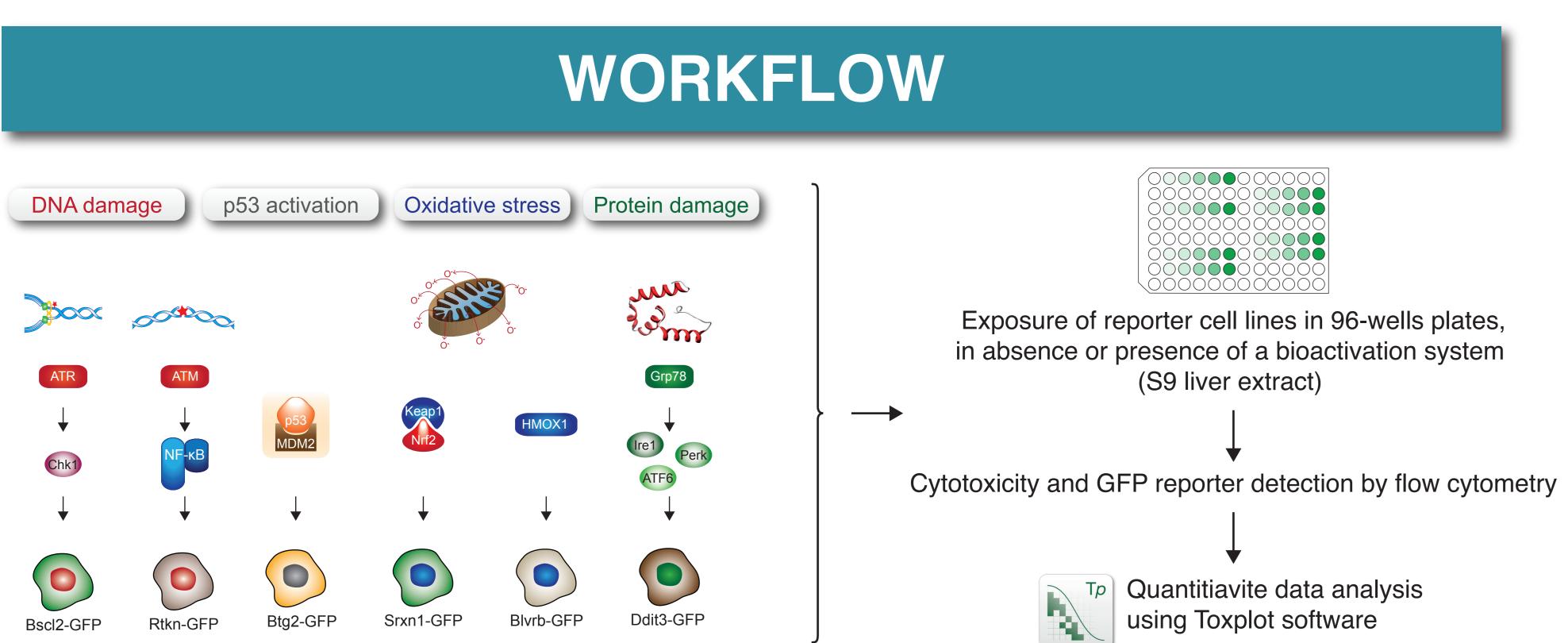
Mechanistic approaches to further underpin these screening assays & increase their predictivity.





OBJECTIVES

Investigate whether the ToxTracker assay can be applied to petroleum UVCBs as a high-content screen for mutagenicity / carcinogenicity by testing by testing twelve DMSO extracts of petroleum substances for (geno)toxic properties in the assay.



MATERIALS & METHODS

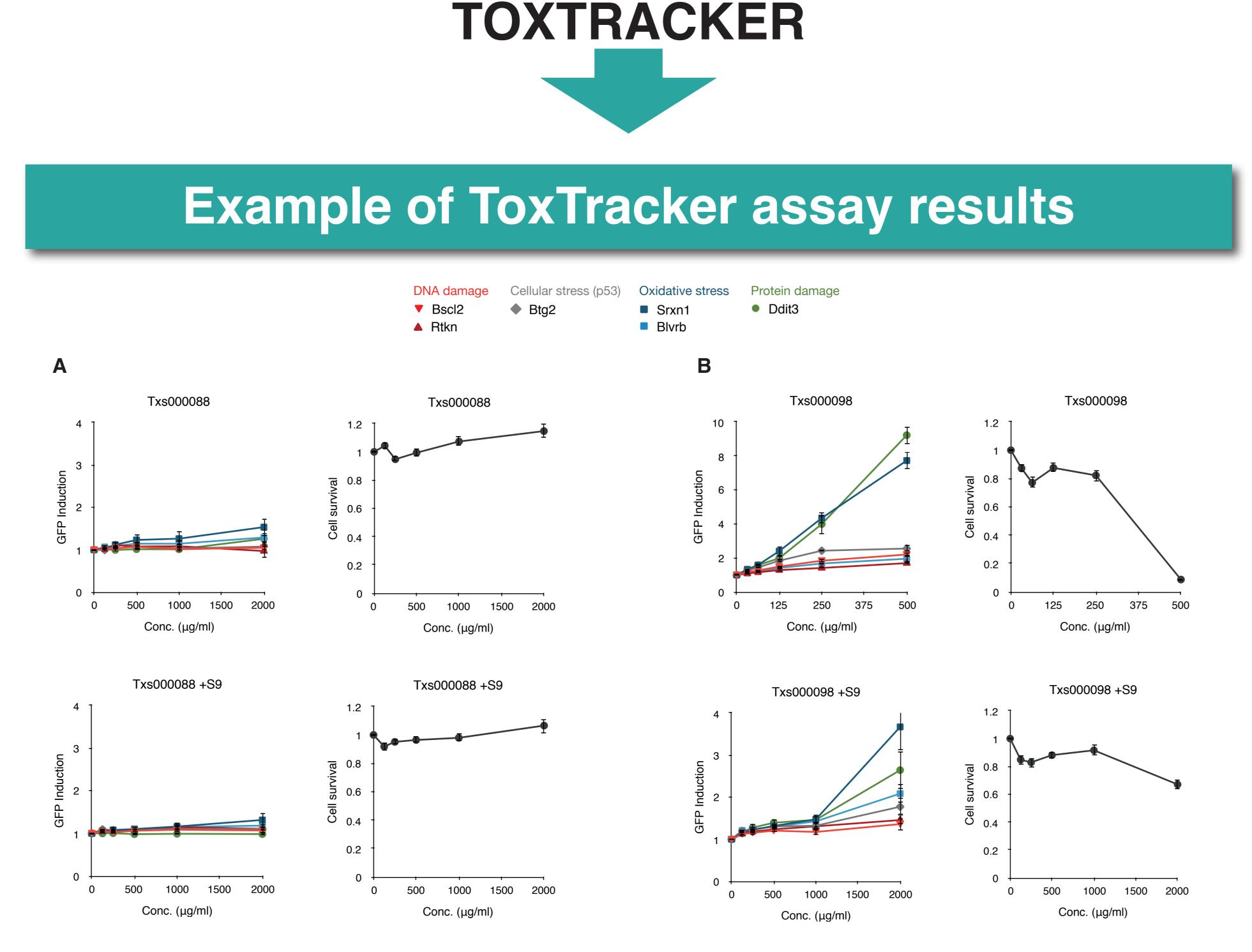
ToxTracker is a panel of mammalian stem cell lines that contain different fluorescent reporters for induction of DNA damage, oxidative stress and protein damage. The reproter cell lines were exposed to twelve DMSO extracts of petroleum substances obtained from 4 Concawe member companies. The differential induction of the GFP reporters as well as cytotoxicity of the tested substances was determined by flow cytometry. Exposure to various control compounds were included in each test to confirm acceptable technical performance and reproducibility of the ToxTracker assay. Quantitive data analysis is done using ToxPlot software

- DMSO extracts, containing the "biologically active" fraction (i.e. aromatics) of the petroleum substances were prepared following standard procedures (Roy et al, 1988).
- These DMSO extracts were tested in the ToxTracker assay at 5 different levels of cytotoxicity (0-60%), along with 4 positive control samples.
- The DMSO extracts were analysed in the absence and presence of S9 hamster liver extract to include a bioactivation system.
- In parallel, aliquots of these DMSO extracts were tested in the standard Modified Ames test (bacterial reverse mutation test modified according to ASTM E1687-10, specified for testing petroleum substances) for validation purposes and analysed by PAC2 analysis to determine the PAC levels in the samples since 3-7 ring PAC are expected to be associated with the toxicities under investigation in the current assay.".

Validation of the ToxTracker reporter assay for the genetic toxicology assessment of petroleum products

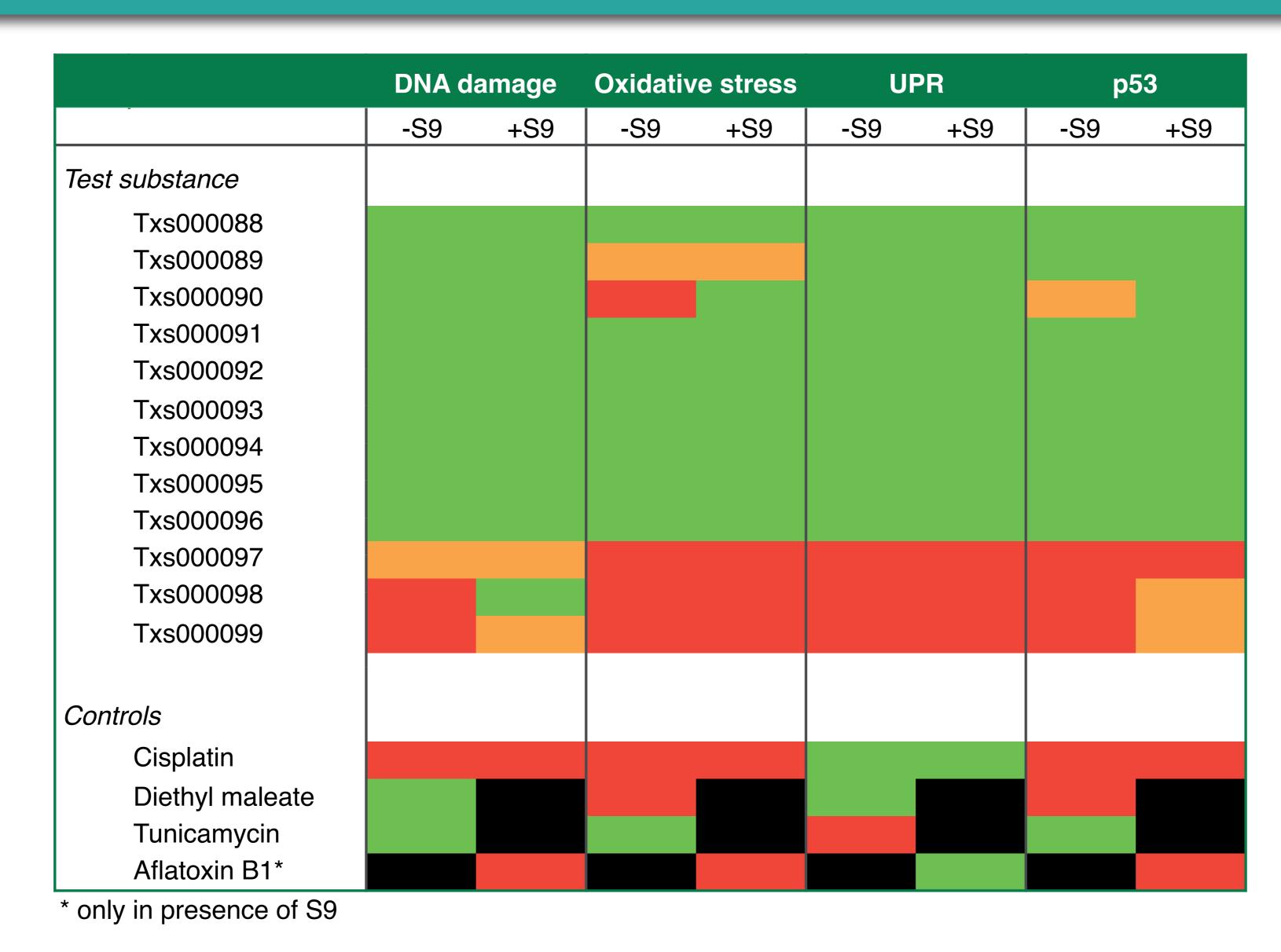
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Example results from the ToxTracker assay. Twelve petroleum substance were tested in the ToxTracker assay for induction of DNA damage, oxidatiuve stress and protein damage (left panel) as well as induction of cytotoxicity (right panel). All substances were analysed in absence and presence S9 hamster liver extract for bioactivation. The figure displayes the Tox Tracker results for A) a refined lubricating oil (Txs000088) and B) a distillate aromatic extract (Txs000098).

Summary of ToxTracker results





Positive (>2-fold induction) Weak positive (1.5 to 2-fold induction) Negative (<1.5-fold induction) Not tested in this study

RESULTS

ANALYTICAL CHEMISTRY

PAC2 analysis

	total Wt.%	Group Percentages of total Wt%							
Sample Identification		I	II	111	IV	V	VI	≥VII	
TXS000088	1.1	0	13	77	8	1	0	0	
TXS000089	1.7	0	1	19	34	31	13	2	
TXS000090	2.8	0	1	1	10	19	33	35	
TXS000091	0	0	0	0	0	0	0	0	
TXS000092	0	0	0	0	0	0	0	0	
TXS000093	0.31	0	7	38	33	16	5	0	
TXS000094	0	0	0	0	0	0	0	0	
TXS000095	0.17	0	5	23	17	17	21	17	
TXS000096	0	0	0	0	0	0	0	0	
TXS000097	9	0	2	1	6	22	36	33	
TXS000098	9.7	0	0	18	49	29	4	0	
TXS000099	12	0	0	22	41	25	11	1	

PAC2 analysis results. The twelve petroleum substances that were tested in this study were analysed for their polycyclic aromatic compounds (PAC) composition. Total wt % of PAC content of the twelve substances are shown in the second column. Group percentages represent the levels of the molecules per aromatic ring class in the twelve tested substances. Results of the two substances used as examples in the Toxtracker results panel on the left are highlighted here as well; the refined lubricating baseoil (TXS000088) has a low level of (mainly 2-3 ring) PACs, whereas the distillate aromatic extract (TXS000098) has a relatively high level of (3-6 ring) PACs.

CONCLUSIONS

- The ToxTracker assay was applicable to complex petroleum UVCBs, including bioactivation by S9 hamster liver extract following standard operation procedures in the currently applied screens in the petroleum industry (Modified Ames and IP346)
- Petroleum extracts with a high PAC content activated the genotoxicity reporters in the ToxTracker assay
- ToxTracker indicated a mutagenic rather then a clastogenic mode-of-action of the genotoxic petroleum extracts.
- The genotoxicity results from ToxTracker showed a good correlation with mutation induction in the ModAmes test
- Overall, the current results from the Tracker assay applied to petroleum streams low in PAC content have a low potential to be mutagenic / carcinogenic in contrast to petroleum streams with relatively high level PAC
- This study is a promising first step towards developing a novel assay to screen for the mutagenic / carcinogenic potential of petroleum streams
- Future work includes investigating ways to prevent auto-fluorescence of PACs in substances with higher PAC levels than presented in the current study

REFERENCES

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- Hendriks G, van de Water B, Schoonen W, Vrieling H. (2013). Cellular-signaling pathways unveil the carcinogenic potential of chemicals. J Appl Toxicol. 33, 399–409.
- Hendriks G, Derr RS, Misovic B, Morolli B, Calléja FMGR, Vrieling H. (2016). The Extended ToxTracker Assay Discriminates Between Induction of DNA Damage, Oxidative Stress, and Protein Misfolding. Toxicol Sci. 150, 190–203.



MODIFIED AMES

Modified Ames test results

	ModAmes (MI)						
Sample Identification		with S9,					
	with S9	lower	without S9				
		CONC.					
TXS000088	0						
TXS000089	1.57		1.69				
TXS000090	0.31		MCe				
TXS000091	0						
TXS000092	0.04						
TXS000093	0						
TXS000094	0.13						
TXS000095	0.11						
TXS000096	0						
TXS000097	1.63	1.93	MCe				
TXS000098	2.82	2.16	MC e				
TXS000099	1.69	2.22	MCe				

Mutation induction by the petroleum products. Modified Ames tests were conducted on the twelve petroleum substances teste in the Toxtracker assay to analyse their ability to induce gene mutations. Mutation Indices (MIs) are shown for all twelve substances. As defined in ASTM E 1687, MI values <1 are considered to have a high probability of being non-carcinogenic in a mouse skin painting bio-assay, values >1 but <2 may or may not be non-carcinogenic in a mouse skin painting assay, whereas values >2 are considered to have a high probability of being positive in a mouse skin painting bio-assay. Results of the two substances used as examples in the Toxtracker results panel on the left are highlighted here as well; the refined lubricating baseoil (TXS000088) has an MI<1, whereas the distillate aromatic extract (TXS000098) has an MI>2.

ACKNOWLEDGEMENTS

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