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BTO report

Bioassays als vervanging voor chemische analyses?

BTO

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BTO *Management samenvatting*

*ToxCast/Tox21 bioassays **geen** vervanging voor huidige chemische analyses van somparameters uit het Drinkwaterbesluit*

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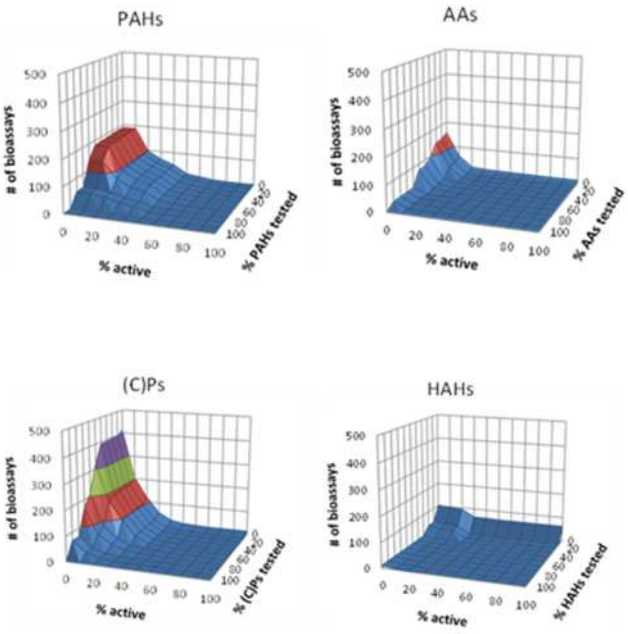
ToxCast/Tox21 in vitro bioassays zijn niet geschikt om de totale concentratie van polycyclische koolwaterstoffen (PAKs), (chloor)fenolen ((C)Fs), aromatische amines (AAs) en gehalogeneerde alifatische koolwaterstoffen (GAKs) in water te bepalen. Dat blijkt uit een studie waarin gegevens uit een groot aantal bioassays zijn geanalyseerd om de bruikbaarheid van bioassays te evalueren als alternatief voor de chemisch-analytische methodes die tot op heden worden ingezet voor het vaststellen van somparameters. Het Drinkwaterbesluit stelt dat voor een goede kwaliteitsgarantie waterbedrijven verplicht zijn hun drinkwater(bronnen) te monitoren volgens vastgestelde normen en signaleringsparameters. Deze studie onderzoekt of hiervoor in vitro bioassays kunnen worden ingezet. Voor dit onderzoek is de ToxCast/Tox21 database van de US-EPA geraadpleegd, waarin gegevens van 9.000 stoffen in meer dan 1.000 bioassays staan. Het onderzoek laat zien dat bioassays niet geschikt zijn om de totale concentratie van AAs en GAKs in water te bepalen, omdat deze stoffen niet tot nauwelijks actief zijn in de bioassays. Ook zijn bioassays niet geschikt om de totale concentratie van PAKs en (C)Fs in water te bepalen, omdat er geen directe relatie is tussen de concentratie en de activiteit van deze stoffen in de bioassays.



Aantal bioassays waarin de stoffen uit de somparameters getest zijn (%) en het percentage stoffen dat actief is. AAs: aromatische amines; PAHs: polycyclische aromatische koolwaterstoffen; (C)Ps: (chloor)fenolen; HAHs: gehalogeneerde aromatische koolwaterstoffen. Bijzonder lage percentages kennen de AAs en HAHs, terwijl de waarden van de (C)Ps en PAHs wel hoger zijn maar laag scoren in de beoordeling van de somparameters.

Belang: nagaan of *in vitro* bioassays geschikt zijn voor meten somparameters

Voor een goede kwaliteitsgarantie moeten waterbedrijven hun drinkwater regelmatig monitoren. Volgens het Drinkwaterbesluit horen specifieke (groepen) stoffen te voldoen aan vastgestelde normen (Tabel II van het Drinkwaterbesluit). Daarnaast zijn signaleringsparameters opgenomen voor groepen stoffen (Tabel IIIc van het Drinkwaterbesluit). Naast het gebruik van chemisch-analytische methoden om na te gaan of drinkwater aan de normen voldoet en of signaleringsparameters niet worden overschreden, hebben waterbedrijven belangstelling voor de inzet van (*in vitro*) bioassays voor waterkwaliteitsmonitoring als mogelijk sneller en/of goedkoper alternatief. In de huidige studie is voor een selectie van de somparameters uit het Drinkwaterbesluit onderzocht of *in vitro* bioassays volstaan als alternatieve methode voor de chemisch-analytische bepaling van de totale concentratie aan stoffen.

Aanpak: analyse van gegevens uit de ToxCast/Tox21 database en prioritering van bioassays

Eerst is onderzocht of over de stoffen die Vitens meet gegevens voorkomen in de ToxCast/Tox21 database van de US EPA (https://actor.epa.gov/dashboard/) en is geïnventariseerd welke gegevens dat zijn. De ToxCast/Tox21 database bevat gegevens van 9.000 stoffen in meer dan 1.000 high-throughput bioassays. Gekeken is naar stoffen die behoren tot de somparameters polycyclische aromatische koolwaterstoffen (PAKs), aromatische amines (AAs), (chloor)fenolen ((C)Fs) en gehalogeneerde alifatische koolwaterstoffen (GAKs). Vervolgens is geanalyseerd welke bioassays leiden tot een respons, met een onderscheid tussen stoffen die wel of niet behoren tot de somparameters. Hier was het doel om tot een selectie van 10 bioassays per somparameter te komen, gebaseerd op scores voor i) inclusiviteit, ii) responsiviteit en iii) specificiteit. Tot slot is nagegaan of de geselecteerde bioassays gevoelig genoeg zijn om de stoffen in water te kunnen meten bij concentraties zoals genoemd in het Drinkwaterbesluit voor de somparameters.

Resultaten: bioassays geven geen respons, of zijn onvoldoende gevoelig en niet specifiek

Uit het onderzoek blijkt dat AAs en GAKs in weinig bioassays een respons geven. PAKs en (C)Fs zijn wel in een aantal bioassays actief, maar voor verschillende stoffen binnen een somparameter zijn verschillende concentraties (µg/L) nodig om een effect te geven. Geen enkele bioassay is in staat om alle stoffen uit de somparameters te detecteren. Daarnaast zijn de bioassays voor PAKs en (C)Fs niet specifiek en onvoldoende gevoelig om stoffen te detecteren bij concentraties die genoemd staan in het Drinkwaterbesluit voor de somparameters.

Implementatie: bioassays niet geschikt als vervanging chemische analyse somparameters

Op grond van de resultaten van dit onderzoek luidt de conclusie dat de ToxCast/Tox21 bioassays niet geschikt zijn om de chemische analyse van geselecteerde somparameters te vervangen om de totale concentratie (µg/L) stoffen in water te bepalen. Daarom zijn bioassays niet geschikt om na te gaan of water voldoet aan de huidige normen of grenzen van signaleringsparameters voor somparameters uit het Drinkwaterbesluit.

Rapport

Dit onderzoek is verschenen als *peer review* artikel in: Chemosphere 209 (2018) pp. 373-380 (https://doi.org/10.1016/j.chemosphere.2018.06.056) en met de Nederlandse conclusies samengevoegd in rapport BTO 2018.075 met als titel "*Bioassays als vervanging voor chemische analyses?*".

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Summary

Note: abstract is taken from Louisse et al., (2018): article in Chemosphere 209.

The present study assesses whether the high throughput ToxCast and Tox21 bioassays for which information and effect data are available via the iCSS ToxCast Dashboard provided by the US EPA, are suitable as candidate bioassays to be used for water quality monitoring of groups of chemicals (sum parameters) as defined in the European and Dutch Drinking Water Directives (DWD).

To this aim, the ToxCast/Tox21 database was explored for bioassays that can detect effects of polycyclic aromatic hydrocarbons (PAHs), which are included in the Dutch and European DWD as a sum parameter, and aromatic amines (AAs), (chloro)phenols ((C)Ps) and halogenated aliphatic hydrocarbons (HAHs), which are included as sum parameters in the Dutch DWD.

Based on the analysis of the availability and performance of bioassays included in the ToxCast/Tox21 database, we concluded that several bioassays are suitable as bioanalytical tools for assessing the presence of PAHs and (C)Ps in drinking water sources. Such a conclusion could not be drawn for AAs and HAHs, due to the limited activity of these chemicals in the bioassays and the limited amount of data on these chemicals in the database. The analysis also indicates that no individual bioassay exists in the ToxCast/Tox21 database that detects all PAHs and/or all (C)Ps, and no bioassay is specific for only PAHs or (C)Ps.

To apply the available bioassays in water quality monitoring, e.g. complementary to chemical analysis, the sensitivity of the identified ToxCast/Tox21 bioassays needs to be improved.

Contents

1 Manuscript (Engels)

De tekst in dit hoofdstuk is verschenen als peer review artikel in: Chemosphere 209 (2018) pp. 373- 380. DOI link: https://doi.org/10.1016/j.chemosphere.2018.06.056

1.1 Introduction

In the EU, drinking water and its sources are regularly monitored to assess compliance with microbial and chemical standards. The chemical parameters in the European Drinking Water Directive (DWD) include statutory standards and indicator parameters for individual chemicals as well as statutory standards for groups of chemicals, such as polycyclic aromatic hydrocarbons (PAHs) and pesticides (Council Directive 98/83/EC). In the Dutch DWD, these are complemented with indicator parameters for groups of chemicals, such as aromatic amines (AAs), (chloro)phenols ((C)Ps) and halogenated aliphatic hydrocarbons (HAHs). In the standards for groups of chemicals (i.e. sum parameters) the maximum summed concentration allowed in drinking water is defined, e.g. 0.1 µg/L for a selection of PAHs. Drinking water companies have extensive monitoring programs to determine chemical concentrations in drinking water and its sources, to comply with national legislation and to be able to manage potential health risks. Targeted analytical chemical techniques are routinely used to measure the sum parameters in these monitoring programs, however these only cover a pre-selected set of chemicals. With this approach, other chemicals may go unnoticed, including emerging chemicals that entered the aquatic environment recently, as well as chemicals that cannot be detected due to limitations in the applied analytical techniques.

In vitro bioassays have been applied as bioanalytical tools to obtain information on the chemical quality of drinking water and its sources (Brand et al., 2013; Kolkman et al., 2013; Escher et al., 2014; Escher et al., 2015; Di Paolo et al., 2016; König et al., 2017; Leusch et al., 2017). In vitro bioassays integrate the total biological response of the mixture of known and unknown chemicals present in a (water)sample. At present, important challenges for implementing in vitro bioassays in water quality monitoring are related to the selection and interpretation of bioassays (Schriks et al., 2015; Dingemans et al., in preparation) and legal embedding of the bioassays in the EU DWD and Water Framework Directive (WFD, Brack et al., 2017).

The present study investigates whether the high throughput EPA ToxCast and Tox21 databases (https://actor.epa.gov/dashboard) can provide information to select candidate bioassays to measure sum parameters. To this aim, in vitro data on all bioassays in the database were explored for chemicals that are included in selected sum parameters derived from the Dutch DWD. The sum parameters selected in the present study were PAHs, for which guideline values have been defined in the European and Dutch DWD, and AAs, (C)Ps and HAHs, for which indicator parameters have been defined in the Dutch DWD.

1.2 Methods

We first assessed which chemicals that are measured to monitor the sum parameters PAHs, AAs, (C)Ps and HAHs (Supplementary Tables 1-4), are present in the ToxCast/Tox21 database. The ToxCast and Tox21 data that are available via the iCSS ToxCast Dashboard consist of 1196 assay endpoints. The majority of the bioassays applied in ToxCast/Tox21 give information on a single assay endpoint, but some bioassays can be used to test effects on different endpoints simultaneously. In this study, the term 'bioassay' is used for all assay endpoints in cellular and biochemical assays included in the ToxCast/Tox21 database. Since not all chemicals included in the ToxCast/Tox21 studies (>9000) have been tested in all bioassays, we assessed for each bioassay whether the chemicals included in the sum parameters of interest have been tested and whether the tested chemicals are active (i.e. a 50% activity concentration (AC50) is available). Bioassays in which the majority of chemicals of the respective sum parameter have not been tested or are inactive at the highest tested concentration are considered to have little potential for inclusion in routine monitoring of drinking water sources. Also, if chemicals that are not related to a sum parameter are active in a bioassay, this bioassay is considered less suitable for measuring the specific sum parameter. We first made for each sum parameter a preselection of 20 possibly relevant bioassays based on the highest number of active chemicals of the sum parameter. The performance of these preselected bioassays was subsequently evaluated in more detail on i) inclusiveness, ii) responsiveness and iii) specificity for the specific sum parameter. These parameters are considered of equal importance and represent: i) the number of active chemicals of the sum parameter as a percentage of the number of chemicals of the sum parameter and ii) the number of active chemicals of the sum parameter as a percentage of the number of the chemicals of the sum parameter that has been tested, and iii) the number of the active chemicals of the sum parameter as a percentage of all active chemicals in that bioassay as reported in the ToxCast/Tox21 database. The sensitivity of the selected bioassays was assessed by calculating the average active concentration, which is the average concentration of the active chemicals of the sum parameter in water that is required to evoke a response (50% of maximum response) in the bioassay (using an arbitrary Relative Enrichment Factor (REF) of 100). This information on sensitivity was not used to rank bioassays, since the sensitivity of bioassays is chemical-specific and AC50 values for different chemicals of a sum parameter within a single bioassay sometimes differ two orders of magnitude. However, bioassays with an average active concentration higher than 100 µg/L were excluded for further analysis, because their sensitivity was regarded to be too low. Based on these analyses 10 bioassays were selected per sum parameter as having the highest potential to be used in water quality monitoring for the sum parameters.

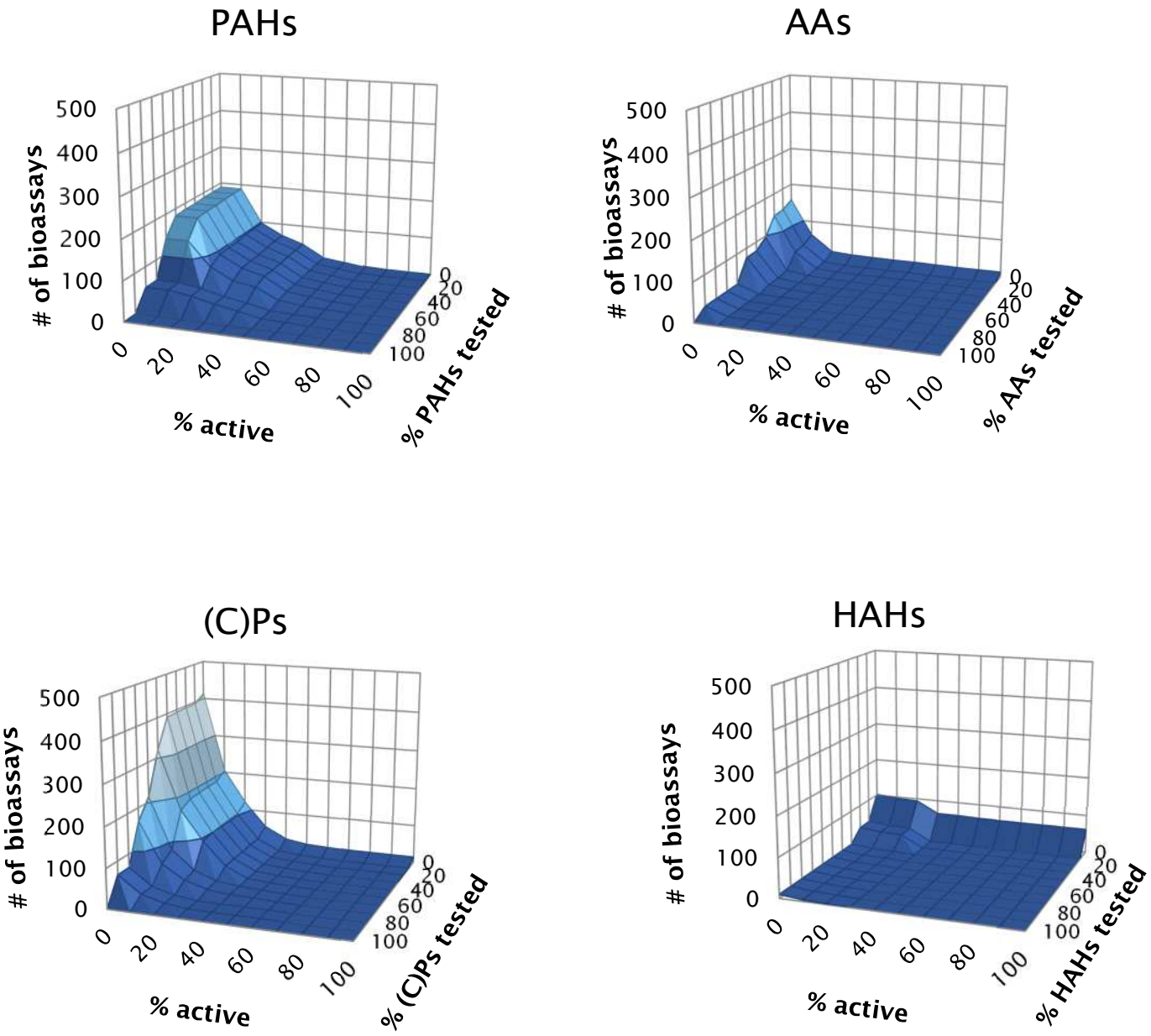

Figure 1. Number of ToxCast/Tox21 bioassays as a function of the percentage of chemicals included in the sum parameter that has been tested and of the percentage of tested chemicals included in the sum parameter that is active.

1.3 Results and Discussion

For the PAH sum parameter, 4 individual PAHs have been defined in the European DWD and 10 in the Dutch DWD, whereas for the Dutch indicator parameters for AAs, (C)Ps and HAHs individual chemicals have not been defined. In practice, drinking water companies have implemented their own selection of target chemicals that cover specific parameters. The ToxCast/Tox21 database contains data for 10 PAHs, 35 AAs, 42 (C)Ps and 15 HAHs (being 100%, 76%, 91% and 94% of the sum parameter members routinely monitored by Vitens, a Dutch drinking water company) (Supplementary Tables 1-4 and Supplementary Figures 1-4). Figure 1 shows for each sum parameter the number of ToxCast/Tox21 bioassays as a function of the percentage of chemicals included in the sum parameter that has been tested and of the percentage of tested chemicals included in the sum parameter that is active. In general, the number of bioassays in which chemicals included in the sum parameters are active is low, especially for the AAs and HAHs. Besides, HAHs have been tested in relatively few bioassays. Therefore, AAs and HAHs are not further explored in the present study. For PAHs and (C)Ps, a priority list of 20 bioassays based on the number of active chemicals was defined for further evaluation (Supplementary Tables 5 and 6). From these priority lists, bioassays were removed in which the average active concentration is higher than 100 µg/L (Supplementary Table 5, bioassays 7-10). Also, bioassay endpoints were removed that cannot be considered as standalone readouts (e.g. endpoints used for background signal correction (Supplementary Table 5, bioassays 1, 5, 16-19)).

For the PAHs, data obtained for the 20 preselected bioassays are presented in Supplementary Table 5 and for the 10 selected bioassays in Table 1. Background information on the 10 selected bioassays is presented in Supplementary Table 7. There is no individual bioassay that detects effects of all 10 PAHs. Table 1 summarizes the performance of the selected bioassays based on i) inclusiveness, ii) responsiveness and iii) specificity for the sum parameter PAHs and presents the average active concentrations. The scores on inclusiveness range between 40% and 60% and the scores on responsiveness between 50 and 67%. The specificity of the 10 selected bioassays is low (between 0.3% and 3.2%), indicating that many other chemicals are active in the bioassays. Therefore, other chemicals than PAHs present in water may also provoke a response when applied in water quality monitoring. When adding up the scores for the three criteria the TOX21_ESRE_BLA_ratio bioassay (Table 1, bioassay A) is the most promising bioassay, followed by the CEETOX_H295R_OHPROG_dn bioassay (Table 1, bioassay B) and the ATG_PXRE_CIS_up bioassay (Table 1, bioassay C). However, for all 10 bioassays, average active concentrations are between 24 and 74 µg/L (Table 1) which are 240-740 times higher than the drinking water standard of 0.10 µg/L for PAHs, indicating that the bioassays are not sensitive enough to be applied in water quality monitoring to detect PAHs.

Table 1. Overview of activity of PAHs in 10 selected ToxCast/Tox21 bioassays (A-J). Pink-red: active chemical; blue: no effect observed; blank: chemical has not been tested. For bioassays in which a response is observed, the mass concentration in a water sample (µg/L) is given that would give a 50% effect in the bioassay, assuming a REF of 100. For each bioassay information on the performance, according to the criteria for i) inclusiveness, ii) responsiveness and iii) specificity are presented as well. The greener the box, the higher the value for that criterium compared with the other bioassays. Also, for each bioassay, the average active concentration (average concentration (µg/L) of the active PAHs in a water sample that is required to evoke a response (50% of maximum) in the bioassay, applying a REF of 100) is presented.

PAH	**CAS**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Anthracene	120-12-7	81									
Benz(a)anthracene	56-55-3	88	14	18	31	13	6.3			0.041	113
Benzo(b)fluoranthene	205-99-2	86	10		2.7			6.9	33		94
Benzo(k)fluoranthene	207-08-9	7.2			5.2						28
Benzo(g,h,i)perylene	191-24-2	11			7.5						
Chrysene	218-01-9				74						
Fluoranthene	206-44-0	172	61	48		73	85	64	58	57	105
Indeno(1,2,3-cd)pyrene	193-39-5			4.3							
Phenanthrene	85-01-8		67	99		93	107	79	80	35	
Pyrene	129-00-0		54	19		53	65	51	62	44	
# of positive PAHs		6	5	5	5	4	4	4	4	4	4
Evaluation citeria											
i) inclusiveness (%)		60	50	50	50	40	40	40	40	40	40
ii) responsiveness (%)		67	63	63	56	50	50	50	50	50	44
iii) specificity (%)		1.7	3.2	0.3	0.3	2.6	2.1	1.9	0.7	0.6	0.8
Sum evaluation criteria i-iii		128	116	113	106	93	92	92	91	91	85
average active effect concentration water		74	41	38	24	58	66	50	58	34	85

A. TOX21_ESRE_BLA_ratio
B. CEETOX_H295R_OHPROG_dn
C. ATG_PXRE_CIS_up
D. TOX21_ARE_BLA_agonist_ratio
E. CEETOX_H295R_TESTO_dn
F. CEETOX_H295R_ANDR_dn
G. CEETOX_H295R_11DCORT_dn
H. ATG_RXRb_TRANS_up
I. BSK_3C_Proliferation_down

TOX21_ERa_BLA_Agonist_ratio

Table 2. Overview of activity of (C)Ps in 10 selected ToxCast/Tox21 bioassays (A-J). Pink-red: active chemical; blue: no effect observed; blank: chemical has not been tested. For bioassays in which a response is observed, the mass concentration in a water sample (µg/L) is given that would give a 50% effect in the bioassay, assuming a REF of 100. For each bioassay information on the performance, according to the criteria for i) inclusiveness, ii) responsiveness and iii) specificity are presented as well. The greener the box, the higher the value for that criterium compared with the other bioassays. Also, for each bioassay, the average active concentration (average concentration (µg/L) of the active (C)Ps in a water sample that is required to evoke a response (50% of maximum) in the bioassay, applying a REF of 100) is presented.

(C)P	CAS	A	B	C	D	E	F	G	H	I	J
Bisphenol A	80-05-7	47	143	0.27	56	3.4	0.22	0.87	0.73	29	90
2-Ethylphenol	90-00-6				46	20					
3-Ethylphenol	620-17-7					33					99
4-Ethylphenol	123-07-9			90			200		66		
4-Chloro-2-methylphenol	1570-64-5	75		82	49		107	113	73		
4-Chloro-3-methylphenol	59-50-7	60	151	57			70	67	72		
2-Chlorophenol	95-57-8				83						
3-Chlorophenol	108-43-0	71									
4-Chlorophenol	106-48-9			88			210	74	42		
m-Cresol	108-39-4										
o-Cresol	95-48-7									5.2	
p-Cresol	106-44-5									7.7	
2,3-Dichlorophenol	576-24-9	81									
2,4-Dichlorophenol	120-83-2	56	90	107	58	109	133	113	61		
2,5-Dichlorophenol	583-78-8	58				36					
2,6-Dichlorophenol	87-65-0										
3,4-Dichlorophenol	95-77-2	16									31
3,5-Dichlorophenol	591-35-5	17									
2,3-Dimethylphenol	526-75-0			56		26	72			1.9	
2,4-Dimethylphenol	105-67-9			127	9.7					4.5	
2,5-Dimethylphenol	95-87-4									9.9	
2,6-Dimethylphenol	576-26-1			37						5.2	
3,4-Dimethylphenol	95-65-8			92	85		200			7.1	
4-Nonylphenol	104-40-5	20	131	8.6	21	25	4.8	23	30	20	109
4-Octylphenol	1806-26-4	19	9.9	5.4	23	17	4.6	21	23	7.2	129
Pentachlorophenol	87-86-5	4.6	1.7		26	15					33
4-Pentylphenol	14938-35-3			2.2	33	54	3.3			5.3	
Phenol	108-95-2										
2,3,4,5-Tetrachlorophenol	4901-51-3	12									17
2,3,4,6-Tetrachlorophenol	58-90-2	31			50	57					67
2,3,5,6-Tetrachlorophenol	935-95-5	19									85
4-(1,1,3,3-Tetramethylbutyl)phenol	140-66-9	9.5	8.8	2.3	15	4.1	3.8	1.5	4.6		50
2-tert-Butylphenol	88-18-6	90		27	59			104			
3-tert-Butylphenol	585-34-2	75		21		32	11	61	65		
4-tert-Butylphenol	98-54-4	56	85	2.5	166	35	6.7	54	48	4.8	
2,3,4-Trichlorophenol	15950-66-0	13									130
2,3,5-Trichlorophenol	933-78-8	14									156
2,3,6-Trichlorophenol	933-75-5	94				121					111
2,4,5-Trichlorophenol	95-95-4	8.5	9.6	58	60	42	73	94	72		123
2,4,6-Trichlorophenol	88-06-2	114	8.9	133	180	109					57
3,4,5-Trichlorophenol	609-19-8	7.9									27
2,4,6-Tris(tert-butyl)phenol	732-26-3	87	40		150	7.2	3.6	77	153		
# of positive (C)Ps		26	11	19	18	18	16	13	13	12	16
Evaluation citeria											
i) inclusiveness (%)		62	26	45	43	43	38	31	31	29	38
ii) responsiveness (%)		63	100	59	56	56	50	45	45	48	39
iii) specificity (%)		2.8	2.2	2.4	1.3	1.0	1.7	6.0	5.7	3.9	0.9
Sum evaluation criteria i-iii		128	128	107	100	100	90	82	81	80	78
average active effect concentration water		27	16	24	28	18	26	19	17	3	31

A. TOX21_MMP_ratio_down
B. NHEERL_ZF_144hpf_TERATOSCORE_up
C. ATG_ERa_TRANS_up
D. ATG_NRF2_ARE_CIS_up
E. ATG_PXRE_CIS_up
F. ATG_ERE_CIS_up
G. OT_ER_ERbERb_0480
H. OT_ER_ERaERb_0480
I. BSK_LPS_PGE2_down

TOX21_ARE_BLA_agonist_ratio

For the (C)Ps, data obtained for the 20 preselected bioassays are presented in Supplementary Table 6 and for the 10 selected bioassays in Table 2. Background information on the 10 selected bioassays is presented in Supplementary Table 8. There is no single bioassay that detects all 42 (C)Ps. Table 2 shows the performance of the selected bioassays based on i) inclusiveness, ii) responsiveness and iii) specificity for the sum parameter (C)Ps and presents the average active concentrations. The scores on inclusiveness ranges between 26% and 62% and the scores on responsiveness between 39% and 100%. The specificity of the 10 selected bioassays is low (between 0.9% and 6.0%), indicating that many other chemicals are active in the bioassays. Therefore, other chemicals than (C)Ps present in water may provoke a response when applied in water quality monitoring. When adding up the scores for the three criteria the TOX21_MMP_ratio_down bioassay (Table 2, bioassay A) is the most promising bioassay, followed by the ATG_ERa_TRANS_up bioassay (Table 2, bioassay B) and the ATG_NRF2_ARE_CIS_up bioassay (Table 2, bioassay C). However, for the selected 10 bioassays, average active concentrations in water samples are between 3 and 31 µg/L (Table 2) which are 30-310 times higher than the indicator parameter value of 0.10 µg/L for (C)Ps, indicating that the bioassays are not sensitive enough to be applied in water quality monitoring to detect (C)Ps.

The present study shows that bioassays included in the ToxCast/Tox21 database can be used as bioanalytical tools to assess the presence of PAHs and (C)Ps. Insufficient data are available to evaluate the use of bioanalytical tools to measure AAs and HAHs. Although PAHs and (C)Ps are active in a number of ToxCast/Tox21 bioassays, no individual bioassay exists that detects all PAHs and/or all (C)Ps, and no bioassay is specific for only PAHs or (C)Ps. Interestingly, the PXRE_CIS_up bioassay and the TOX21_ARE_BLA_agonist_ratio bioassay were selected in the priority list for both PAHs (Table 1) and (C)Ps (Table 2), so these bioassays can be used to detect both PAHs and (C)Ps. It is important to note that chemicals may have different potencies, and therefore a response of a chemical mixture as determined in a bioassay cannot directly be translated to summed mass concentrations that are set as guideline values or indicator parameters for a sum parameter (i.e. 0.10 µg/L for PAHs and 0.10 µg/L for (C)Ps). Therefore, bioassays cannot be used as a direct replacement of chemical analyses to determine the total concentration of sum parameters. Nevertheless, as the bioanalytical tools integrate potency in their response, we consider them as a better predictor of health risk compared to the summed concentrations of individual chemicals that differ in their potency.

The present study also indicates that the specificity of the bioassays is low for the sum parameters. Therefore, they are not suitable for specifically measuring the sum parameter since other chemicals present may also provoke a response. Also from that perspective bioassays cannot be used as a direct replacement of chemical analyses to determine the total concentration of sum parameters. However, if responses from bioassays can be related to health risks related to various chemicals in water, bioassays that detect many chemicals (instead of only those belonging to a sum parameter) can be considered to be an advantage. In such a monitoring framework based on bioassays, health-based trigger values can be used to evaluate the health relevance of detected bioassay responses (Brand et al., 2013; Escher et al., 2015).

In the context of the present objective, the sensitivity of the bioassays evaluated in this study does not suffice, since they only detect the chemicals at concentrations in water (assuming a REF of 100) that are higher than the drinking water standard of 0.10 µg/L for PAHs and the indicator parameter value of 0.10 µg/L for (C)Ps (Tables 1 and 2). It may however be possible to optimize a bioassay to detect chemicals at lower concentrations by increasing the free concentration of chemicals in the test system. In this regard, the cell model and culture conditions (e.g. cell culture medium) used play an

important role (Groothuis et al., 2015; Fischer et al., 2017). Another strategy is to optimize sample preparation techniques to increase the REF. REFs reported in the literature vary (Escher et al., 2008) and the value of 100 used in the present study may be too low. Further aspects that are of importance for the application of bioassays for water quality monitoring, such as ease of use and costs, have been defined in the EU FP7 project DEMEAU (http://demeau-fp7.eu/).

In conclusion, the present study shows that part of the current collection of ToxCast/Tox21 bioassays can be used to detect PAHs and (C)Ps for drinking water monitoring, but not as bioanalytical tools to determine the total concentration of all chemicals included in the sum parameters of the DWD. Therefore, bioassays cannot be used as a direct replacement of the chemical analyses that are applied to determine compliance with the drinking water standards and indicator parameters. Nevertheless, using a combination of analytical chemical techniques and bioassays in a complementary manner may give an optimal insight in chemical water quality, and the bioassays that emerged in the present study are promising candidates to be used in such a water quality monitoring approach.

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2 Conclusie (Nederlands)

2.1 Haalbaarheid van het vervangen van somparameters door bioassays

De resultaten van het onderzoek tonen aan dat, op basis van de aanwezige informatie, de ToxCast/Tox21 bioassays niet gebruikt kunnen worden als directe vervanging voor de chemische analyse van de geselecteerde somparameters (polycyclische aromatische koolwaterstoffen (PAKs), (chloor)fenolen ((C)Fs), aromatische amines (AAs) en gehalogeneerde alifatische koolwaterstoffen (GAKs)) uit het Drinkwaterbesluit (Dwb).

Deze conclusie is gebaseerd op de volgende bevindingen:

1. De AAs en GAKs zijn niet actief in de meeste ToxCast/Tox21 bioassays waarin ze zijn getest. Aanwezigheid van deze stoffen kan daarom niet worden aangetoond door het inzetten van deze bioassays.
2. PAKs en (C)Fs zijn actief in een aantal bioassays, maar er zijn geen bioassays die alle PAKs en/of alle (C)Fs oppikken.
3. PAKs en (C)Fs zijn actief in een aantal bioassays, maar de potentie van de stoffen die tot dezelfde somparameter behoren verschillen sterk. Daarom kan een effect van een mengsel van stoffen in een bioassay niet direct vertaald worden naar de totale concentratie van deze stoffen, zoals dat nu bepaald moet worden volgens het Dwb.
4. De ToxCast/Tox21 bioassays die PAKs en (C)Fs detecteren zijn niet specifiek voor deze stoffen. Daarom zijn de ToxCast/Tox21 bioassays niet geschikt om de aanwezigheid van specifieke stoffen en/of stofgroepen aan te tonen in complexe mengsels. Dit houdt in dat bij het meten van een watermonster een positieve respons in de bioassay veroorzaakt kan worden door stoffen die niet tot de somparameter behoren.
5. Rekening houdend met een concentratiefactor van 100, is de gevoeligheid van de bioassays die PAKs en (C)Fs detecteren te laag om de stoffen te detecteren bij concentraties die genoemd staan in het Drinkwaterbesluit (norm voor PAKs: 0.10 µg/L; signaleringsparameter voor (C)Fs: 0.10 µg/L).

3 Aanbevelingen (vervolgonderzoek)

Hieronder staat voor elke bevinding aangegeven welke kennislacunes er nog zijn en welke aanbevelingen er zijn voor vervolgonderzoek.

Bevinding 1: De AAs en GAKs zijn niet actief in de meeste ToxCast/Tox21 bioassays waarin ze zijn getest. Aanwezigheid van deze stoffen kan daarom niet worden aangetoond door het inzetten van deze bioassays.

Aangezien de AAs en GAKs slechts zijn getest in een beperkt aantal ToxCast/TOX21 bioassays, zijn er mogelijk wel bioassays beschikbaar waarin deze stoffen wel actief zijn. Echter, het valt niet te verwachten dat er op korte termijn gegevens beschikbaar komen van deze stoffen in de ToxCast/Tox21 bioassays waarin ze nog niet zijn getest. Om te zoeken naar geschikte bioassays die mogelijk alifatische koolwaterstoffen en gehalogeneerde alifatische koolwaterstoffen kunnen detecteren, is het aan te bevelen om de toxicologische werkingsmechanismen van deze stoffen in kaart te brengen (bijvoorbeeld door middel van literatuuronderzoek) en op basis van deze kennis na te gaan of er bioassays beschikbaar zijn die relevant zijn voor deze toxicologische werkingsmechanismen. Indien op basis van dat onderzoek potentieel relevante bioassays beschikbaar zijn, kunnen de stoffen getest worden en kan op basis daarvan worden geconcludeerd of alifatische koolwaterstoffen en/of gehalogeneerde alifatische koolwaterstoffen kunnen worden gedetecteerd met bioassays.

Bevinding 2: PAKs en (C)Fs zijn actief in een aantal bioassays, maar er zijn geen bioassays die alle PAKs en/of alle (C)Fs oppikken.

Aangezien er geen bioassays zijn die alle PAKs of alle (C)Fs oppikken, is het niet mogelijk om voor een somparameter een enkele bioassay in te zetten als alternatief voor de chemische analyses. Om toch alle stoffen binnen een somparameter af te vangen kan een combinatie van bioassays worden gebruikt. Op basis van de ToxCast/Tox21 gegevens blijkt dit mogelijk voor de 10 PAKs genoemd in het Dwb (TOX21_ESRE_BLA_ratio bioassay, ATG_PXRE_CIS_up bioassay en TOX21_ARE_BLA_agonist_ratio bioassay), maar is dit niet mogelijk voor de (C)Fs, omdat een aantal (C)Fs niet actief zijn in de ToxCast/Tox21 bioassays (bijvoorbeeld m-cresol).

Bevinding 3: PAKs en (C)Fs zijn actief in een aantal bioassays, maar de potenties van de stoffen die tot dezelfde somparameter behoren verschillen sterk. Daarom kan een effect van een mengsel van stoffen in een bioassay niet direct vertaald worden naar de totale concentratie van deze stoffen, zoals dat nu bepaald moet worden volgens het Dwb.

Het valt niet te verwachten dat er bioassays beschikbaar zijn waarin alle stoffen van een somparameter dezelfde potentie hebben. Daarom is in het algemeen te stellen dat bioassays niet kunnen worden gebruikt als directe vervanging voor de chemische analyse om concentraties van stoffen behorend tot de geselecteerde somparameters vast te stellen. Er zijn op dit punt geen aanbevelingen voor vervolgonderzoek.

Bevinding 4: De ToxCast/Tox21 bioassays die PAKs en (C)Fs detecteren zijn niet specifiek voor deze stoffen. Daarom zijn de ToxCast/Tox21 bioassays niet geschikt om de aanwezigheid van specifieke stoffen en/of stofgroepen aan te tonen in complexe mengsels. Dit houdt in dat bij het meten van een watermonster een positieve respons in de bioassay veroorzaakt kan worden door stoffen die niet tot de somparameter behoren.

Het valt niet te verwachten dat er bioassays beschikbaar zijn waarin alleen de stoffen van een bepaalde somparameter actief zijn en andere stoffen niet. Daarom kan een respons uit een bioassay waarin een complex mengsel van stoffen aanwezig is niet worden toegeschreven aan alleen stoffen uit de somparameter. Daarom kunnen bioassays niet worden gebruikt als directe vervanging voor de chemische analyse van de geselecteerde somparameters. Er zijn op dit punt geen aanbevelingen voor vervolgonderzoek. Deze bevindingen geven wel aan dat bioassays ingezet zouden kunnen worden om breder te screenen dan alleen stoffen die behoren tot een bepaalde somparameter.

Bevinding 5: De gevoeligheid van de bioassays die PAKs en (C)Fs detecteren is te laag om de stoffen te meten bij relevante concentraties.

De studie laat zien dat de ToxCast/Tox21 bioassays pas effecten meten bij concentraties van de stoffen in water die hoger zijn dan de concentraties die genoemd staan in het Dwb. Bij deze analyse is uitgegaan van een 10000x-concentratie van de stoffen in het water naar een concentraat dat daarna 200 keer verdund wordt voor toepassing in de in vitro bioassays (relative enrichment factor (REF) van 100). Om de bioassays toe te passen in waterkwaliteitsmonitoring is daarom aan te bevelen om te onderzoeken of er mogelijkheden zijn om de stoffen sterker te concentreren. Ook zou uitgezocht kunnen worden of er, qua toxicologische werkingsmechanismen, vergelijkbare in vitro bioassays bestaan die gevoeliger zijn. Het is bijvoorbeeld bekend dat de gevoeligheden van bioassays die oestrogene stoffen detecteren in hoge mate variëren.

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Attachment I Suppl. material

Supplementary Table 1. PAHs as measured by Vitens drinking water company and their presence in the ToxCast/Tox21 dataset.

	PAH	CAS	present in ToxCast/Tox21 dataset
1	Anthracene	120-12-7	yes
2	Benz(a)anthracene	56-55-3	yes
3	Benzo(b)fluoranthene	205-99-2	yes
4	Benzo(k)fluoranthene	207-08-9	yes
5	Benzo(g,h,i)perylene	191-24-2	yes
6	Chrysene	218-01-9	yes
7	Fluoranthene	206-44-0	yes
8	Indeno(1,2,3-cd)pyrene	193-39-5	yes
9	Phenanthrene	85-01-8	yes
10	Pyrene	129-00-0	yes

Supplementary Table 2. AAs as measured by Vitens and presence in the ToxCast/Tox21 dataset.

	AA	CAS	present in ToxCast/Tox21 dataset
1	2'-Aminoacetophenone	551-93-9	yes
2	Aniline	62-53-3	yes
3	2-Anisidine	90-04-0	yes
4	4-Bromoaniline	106-40-1	yes
5	2-Chloroaniline	95-51-2	yes
6	3-Chloroaniline	108-42-9	yes
7	4-Chloroaniline	106-47-8	yes
8	3-Chloro-4-methoxyaniline	5345-54-0	no
9	3-Chloro-4-methylaniline	95-74-9	yes
10	4-Chloro-2-methylaniline	95-69-2	yes
11	5-Chloro-2-methylaniline	95-79-4	yes
12	2,3-Dichloroaniline	608-27-5	no
13	2,4-Dichloroaniline	554-00-7	yes
14	2,5-Dichloroaniline	95-82-9	yes
15	2,6-Dichloroaniline	608-31-1	no
16	3,4-Dichloroaniline	95-76-1	yes
17	3,5-Dichloroaniline	626-43-7	yes
18	Dicloran	99-30-9	yes
19	2,6-Diethylaniline	579-66-8	no
20	N,N-Diethylaniline	91-66-7	yes
21	Dimethadione	695-53-4	yes
22	2,3-Dimethylaniline	87-59-2	yes
23	2,4-Dimethylaniline	95-68-1	yes
24	2,5-Dimethylaniline	95-78-3	yes
25	2,6-Dimethylaniline	87-62-7	yes
26	3,4-Dimethylaniline	95-64-7	yes
27	3,5-Dimethylaniline	108-69-0	yes
28	N,N-Dimethylaniline	121-69-7	yes
29	N-Ethylaniline	103-69-5	yes
30	4-Isopropylaniline	99-88-7	no
31	4-Methoxy-2-nitroaniline	96-96-8	yes
32	3-Methylaniline	108-44-1	yes
33	4-Methylaniline	106-49-0	yes
34	N-Methylaniline	100-61-8	yes
35	4-Methyl-3-nitroaniline	119-32-4	yes
36	2-Nitroaniline	88-74-4	yes
37	3-Nitroaniline	99-09-2	yes
38	Pentachloroaniline	527-20-8	yes
39	2-(Phenylsulfonyl)aniline	4273-98-7	yes
40	2,3,4,5-Tetrachloroaniline	634-83-3	no
41	2,3,5,6-Tetrachloroaniline	3481-20-7	no
42	2,3,4-Trichloroaniline	634-67-3	no
43	2,4,5-Trichloroaniline	636-30-6	no
44	2,4,6-Trichloroaniline	634-93-5	yes
45	3,4,5-Trichloroaniline	634-91-3	no
46	3-Trifluoromethylaniline	98-16-8	no

Supplementary Table 3. (C)Ps as measured by Vitens drinking water company and their presence in the ToxCast/Tox21 dataset.

	(C)P	CAS	present in ToxCast/Tox21 dataset
1	Bisphenol A	80-05-7	yes
2	2-tert-Butylphenol	88-18-6	yes
3	3-tert-Butylphenol	585-34-2	yes
4	4-tert-Butylphenol	98-54-4	yes
5	4-Chloro-2-methylphenol	1570-64-5	yes
6	4-Chloro-3-methylphenol	59-50-7	yes
7	2-Chlorophenol	95-57-8	yes
8	3-Chlorophenol	108-43-0	yes
9	4-Chlorophenol	106-48-9	yes
10	m-Cresol	108-39-4	yes
11	o-Cresol	95-48-7	yes
12	p-Cresol	106-44-5	yes
13	2,3-Dichlorophenol	576-24-9	yes
14	2,4-Dichlorophenol	120-83-2	yes
15	2,5-Dichlorophenol	583-78-8	yes
16	2,6-Dichlorophenol	87-65-0	yes
17	3,4-Dichlorophenol	95-77-2	yes
18	3,5-Dichlorophenol	591-35-5	yes
19	2,3-Dimethylphenol	526-75-0	yes
20	2,4-Dimethylphenol	105-67-9	yes
21	2,5-Dimethylphenol	95-87-4	yes
22	2,6-Dimethylphenol	576-26-1	yes
23	3,4-Dimethylphenol	95-65-8	yes
24	2-Dodecylphenol	5284-29-7	no
25	2-Ethylphenol	90-00-6	yes
26	3-Ethylphenol	620-17-7	yes
27	4-Ethylphenol	123-07-9	yes
28	4-Nonylphenol	104-40-5	yes
29	4-Octylphenol	1806-26-4	yes
30	4-tert-Octylphenol diethoxylate	2315-61-9	no
31	4-tert-Octylphenol monoethoxylate	2315-67-5	no
32	Pentachlorophenol	87-86-5	yes
33	4-Pentylphenol	14938-35-3	yes
34	o-Pentylphenol	136-81-2	no
35	Phenol	108-95-2	yes
36	2,3,4,5-Tetrachlorophenol	4901-51-3	yes
37	2,3,4,6-Tetrachlorophenol	58-90-2	yes
38	2,3,5,6-Tetrachlorophenol	935-95-5	yes
39	4-(1,1,3,3-Tetramethylbutyl)phenol	140-66-9	yes
40	2,3,4-Trichlorophenol	15950-66-0	yes
41	2,3,5-Trichlorophenol	933-78-8	yes
42	2,3,6-Trichlorophenol	933-75-5	yes
43	2,4,5-Trichlorophenol	95-95-4	yes
44	2,4,6-Trichlorophenol	88-06-2	yes
45	3,4,5-Trichlorophenol	609-19-8	yes
46	2,4,6-Tris(tert-butyl)phenol	732-26-3	yes

Supplementary Table 4. HAHs as measured by Vitens drinking water company and their presence in the ToxCast/Tox21 dataset.

	HAH	CAS	present in ToxCast/Tox21 dataset
1	Bromoform	75-25-2	yes
2	Bromochloromethane	74-97-5	yes
3	Bromodichloromethane	75-27-4	yes
4	Carbon tetrachloride	56-23-5	yes
5	Chlorodibromomethane	124-48-1	yes
6	Chloroform	67-66-3	yes
7	Dichloromethane	75-09-2	yes
8	1,1-Dichloroethane	75-34-3	yes
9	1,1-Dichloroethylene	75-35-4	yes
10	(E)-1,2-Dichloroethylene	156-60-5	yes
11	(Z)-1,2-Dichloroethylene	156-59-2	yes
12	Hexachloro-1,3-butadiene	87-68-3	yes
13	Tribromoethylene	598-16-3	no
14	1,1,1-Trichloroethane	71-55-6	yes
15	1,1,2-Trichloroethane	79-00-5	yes
16	1,1,2,2-Tetrachloroethane	79-34-5	yes

Supplementary Table 5. Overview of activity of PAHs in 20 preselected ToxCast/Tox21 bioassays (1-20). Pink-red: active chemical; blue: no effect observed; blank: chemical has not been tested. For bioassays in which a response is observed, the mass concentration in a water sample (µg/L) is given that would give a 50% effect in the bioassay, assuming a REF of 100. For each bioassay information on the performance, according to the criteria for i) inclusiveness, ii) responsiveness and iii) specificity are presented as well. The greener the box, the higher the value for that criterium compared with the other bioassays. Also, for each bioassay, the average active concentration (average concentration (µg/L) of the active PAHs in a water sample that is required to evoke a response (50% of maximum) in the bioassay, applying a REF of 100) is presented.

PAH	CAS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Anthracene	120-12-7	79	81					206	298	223	202							451			
Benz(a)anthracene	56-55-3	89	88	14	18	99	31	50	138	46		13	6.3			0.041	129		75	127	113
Benzo(b)fluoranthene	205-99-2	111	86	10		58	2.7	8.8	31	51	0.22			6.9	33		66	126	72	80	94
Benzo(k)fluoranthene	207-08-9	118	7.2			13	5.2										37	4.2	9.4	111	28
Benzo(g,h,i)perylene	191-24-2	9.2	11			7.0	7.5										7.1	26	5.9	6.2	
Chrysene	218-01-9						74														
Fluoranthene	206-44-0	153	172	61	48	120		225	254	234	242	73	85	64	58	57					105
Indeno(1,2,3-cd)pyrene	193-39-5				4.3																
Phenanthrene	85-01-8			67	99							93	107	79	80	35					
Pyrene	129-00-0			54	19						201	53	65	51	62	44					
# of positive PAHs		6	6	5	5	5	5	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Evaluation citeria																					
i) inclusiveness (%)		60	60	50	50	50	50	40	40	40	40	40	40	40	40	40	40	40	40	40	40
ii) responsiveness (%)		67	67	63	63	56	56	67	67	67	67	50	50	50	50	50	44	44	44	44	44
iii) specificity (%)		5.4	1.7	3.2	0.3	0.6	0.3	1.6	1.3	0.9	1.6	2.6	2.1	1.9	0.7	0.6	6.1	3.7	2.0	0.9	0.8
Sum evaluation criteria i-iii		132	128	116	113	106	106	108	108	108	108	93	92	92	91	91	91	88	86	85	85
average active effect concentration water		93	74	41	38	59	24	122	180	139	161	58	66	50	58	34	60	152	40	81	85

1. TOX21_ESRE_BLA_ch2
2. TOX21_ESRE_BLA_ratio
3. CEETOX_H295R_OHPROG_dn
4. ATG_PXRE_CIS_up
5. TOX21_p53_BLA_p2_ch2
6. TOX21_ARE_BLA_agonist_ratio
7. APR_HepG2_CellCycleArrest_72h_dn
8. APR_HepG2_CellLoss_24h_dn
9. APR_HepG2_CellLoss_72h_dn
10. APR_HepG2_OxidativeStress_72h_up
11. CEETOX_H295R_TESTO_dn
12. CEETOX_H295R_ANDR_dn
13. CEETOX_H295R_11DCORT_dn
14. ATG_RXRb_TRANS_up
15. BSK_3C_Proliferation_down
16. TOX21_VDR_BLA_agonist_ch2
17. TOX21_NFkB_BLA_agonist_ch2
18. TOX21_PPARd_BLA_agonist_ch2
19. TOX21_AR_BLA_Agonist_ch2

TOX21_ERa_BLA_Agonist_ratio

Supplementary Table 6. Overview of activity of (C)Ps in 20 preselected ToxCast/Tox21 bioassays (1-20). Pink-red: active chemical; blue: no effect observed; blank: chemical has not been tested. For bioassays in which a response is observed, the mass concentration in a water sample (µg/L) is given that would give a 50% effect in the bioassay, assuming a REF of 100. For each bioassay information on the performance, according to the criteria for i) inclusiveness, ii) responsiveness and iii) specificity are presented as well. The greener the box, the higher the value for that criterium compared with the other bioassays. Also, for each bioassay, the average active concentration (average concentration (µg/L) of the active (C)Ps in a water sample that is required to evoke a response (50% of maximum) in the bioassay, applying a REF of 100) is presented.

(C)P	CAS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Bisphenol A	80-05-7	47	0.27	56	3.4	0.22	90	0.73	0.87	34	20	29	143	111	70	86	168			1.6	27
2-Ethylphenol	90-00-6			46	20						30									17	
3-Ethylphenol	620-17-7				33		99				69										
4-Ethylphenol	123-07-9		90			200		66													
4-Chloro-2-methylphenol	1570-64-5	75	82	49		107		73	113										155		
4-Chloro-3-methylphenol	59-50-7	60	57			70		72	67				151								
2-Chlorophenol	95-57-8			83																	
3-Chlorophenol	108-43-0	71																			
4-Chlorophenol	106-48-9		88			210		42	74												
m-Cresol	108-39-4																				
o-Cresol	95-48-7											5.2									
p-Cresol	106-44-5											7.7			643						
2,3-Dichlorophenol	576-24-9	81															27				
2,4-Dichlorophenol	120-83-2	56	107	58	109	133		61	113				90								
2,5-Dichlorophenol	583-78-8	58			36																
2,6-Dichlorophenol	87-65-0																				
3,4-Dichlorophenol	95-77-2	16					31			71				103							
3,5-Dichlorophenol	591-35-5	17								126						129	110	132			
2,3-Dimethylphenol	526-75-0		56		26	72						1.9									
2,4-Dimethylphenol	105-67-9		127	10								4.5			200						18
2,5-Dimethylphenol	95-87-4											10									
2,6-Dimethylphenol	576-26-1		37									5.2									
3,4-Dimethylphenol	95-65-8		92	85		200						7.1			164						
4-Nonylphenol	104-40-5	20	8.6	21	25	4.8	109	30	23			20	131	89	17			87	18		13
4-Octylphenol	1806-26-4	19	5.4	23	17	4.6	129	23	21		34	7.2	10	95				128	46	13	12
Pentachlorophenol	87-86-5	4.6		26	15		33			127	22		1.7	22	26	44	6.2	201		10	7.8
4-Pentylphenol	14938-35-3		2.2	33	54	3.3						5.3							52	36	11
Phenol	108-95-2																				
2,3,4,5-Tetrachlorophenol	4901-51-3	12					17			118				31		29	14	109			
2,3,4,6-Tetrachlorophenol	58-90-2	31		50	57		67			196	149			90		72	55			51	30
2,3,5,6-Tetrachlorophenol	935-95-5	19					85			129				51		48	35	210			
4-(1,1,3,3-Tetramethylbutyl)phenol	140-66-9	9.5	2.3	15	4.1	3.8	50	4.6	1.5	137	5.0		8.8	47		89	57	38	13	12	12
2-tert-Butylphenol	88-18-6	90	27	59					104		124				68			80	14	21	
3-tert-Butylphenol	585-34-2	75	21		32	11		65	61		36				78				122	34	
4-tert-Butylphenol	98-54-4	56	2.5	166	35	6.7		48	54		180	4.8	85		195				122	22	
2,3,4-Trichlorophenol	15950-66-0	13					130			110						138					
2,3,5-Trichlorophenol	933-78-8	14					156			121						63	32				
2,3,6-Trichlorophenol	933-75-5	94			121		111														
2,4,5-Trichlorophenol	95-95-4	8.5	58	60	42	73	123	72	94	98	50		10		144				257		23
2,4,6-Trichlorophenol	88-06-2	114	133	180	109		57						8.9								
3,4,5-Trichlorophenol	609-19-8	7.9					27			79				32		55	21	95			
2,4,6-Tris(tert-butyl)phenol	732-26-3	87		150	7.2	3.6		153	77		5.6		40	74	292			339	0.8		23
# of positive (C)Ps		26	19	18	18	16	16	13	13	12	12	12	11	11	11	10	10	10	10	10	10
Evaluation citeria																					
i) inclusiveness (%)		62	45	43	43	38	38	31	31	29	29	29	26	26	26	24	24	24	24	24	24
ii) responsiveness (%)		63	59	56	56	50	39	45	45	29	38	48	100	27	34	24	24	24	31	31	40
iii) specificity (%)		2.8	2.4	1.3	1.0	1.7	0.9	5.7	6.0	1.0	1.2	3.9	2.2	0.6	2.3	3.7	0.9	0.8	1.8	0.9	2.4
Sum evaluation criteria i-iii		128	107	100	100	90	78	81	82	59	67	80	128	54	63	52	49	49	57	56	66
average active effect concentration water		27	24	28	18	26	31	17	19	32	17	3	16	18	45	18	13	34	19	5	4
(C)Ps actief		26	19	18	18	16	16	13	13	12	12	12	11	11	11	10	10	10	10	10	10
(C)Ps getest		41	32	32	32	32	41	29	29	41	32	25	11	41	32	41	41	41	32	32	25
actief		915	785	1362	1802	965	1697	228	218	1206	983	307	500	1816	478	272	1061	1317	563	1053	417

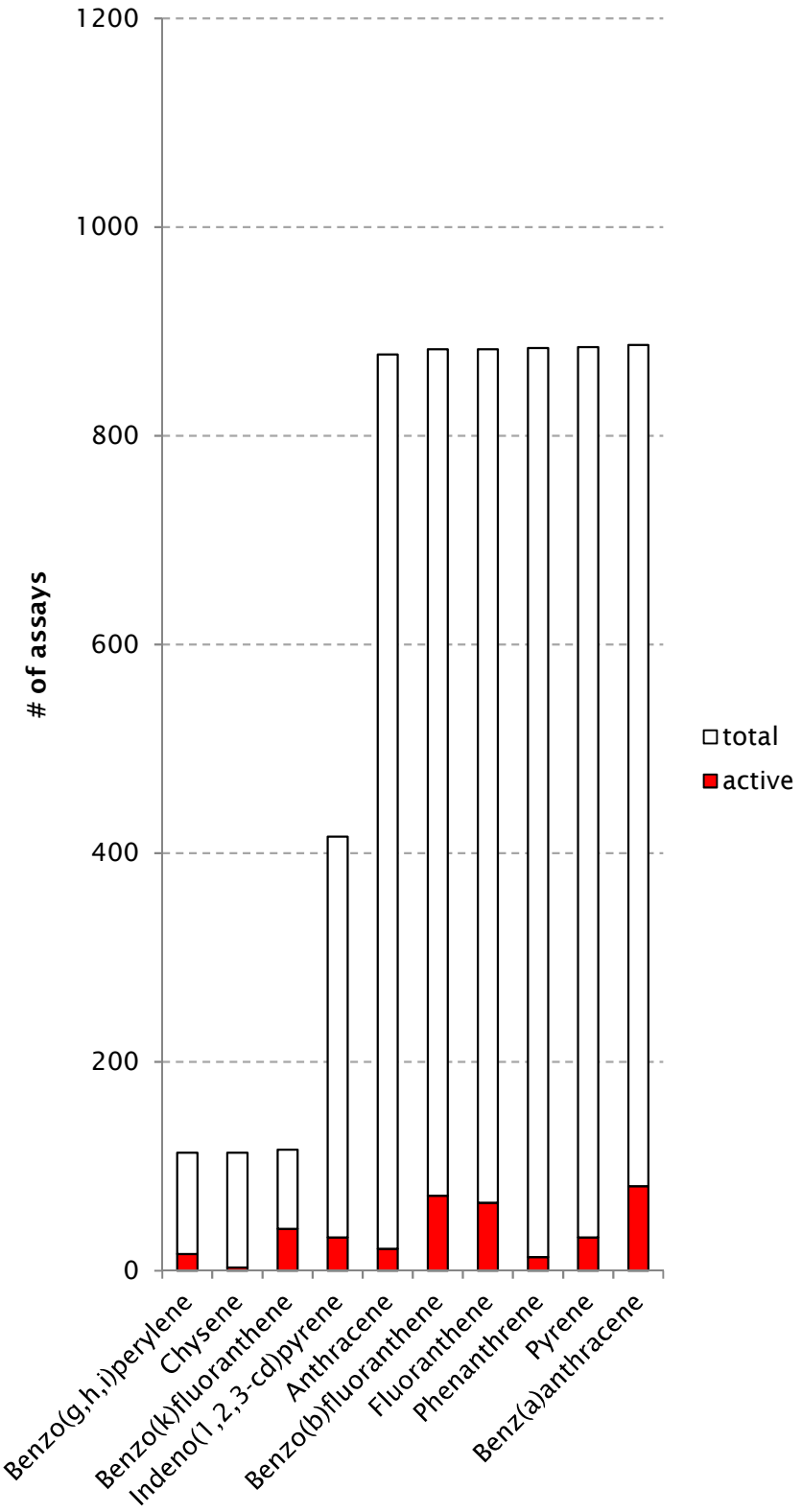
1. TOX21_MMP_ratio_down
2. ATG_ERa_TRANS_up
3. ATG_NRF2_ARE_CIS_up
4. ATG_PXRE_CIS_up
5. ATG_ERE_CIS_up
6. TOX21_ARE_BLA_agonist_ratio
7. OT_ER_ERaERb_0480
8. OT_ER_ERbERb_0480
9. TOX21_AR_LUC_MDAKB2_Antagonist2
10. ATG_VDRE_CIS_up
11. BSK_LPS_PGE2_down
12. NHEERL_ZF_144hpf_TERATOSCORE_up
13. TOX21_TR_LUC_GH3_Antagonist
14. ATG_TCF_b_cat_CIS_dn
15. TOX21_ERa_BLA_Antagonist_ratio
16. TOX21_p53_BLA_p2_ratio
17. TOX21_Aromatase_Inhibition
18. ATG_RXRb_TRANS_up
19. ATG_PXR_TRANS_up
20. BSK_hDFCGF_Proliferation_down

Supplementary Table 7. Background information on the 10 selected ToxCast/Tox21 bioassays for PAHs.

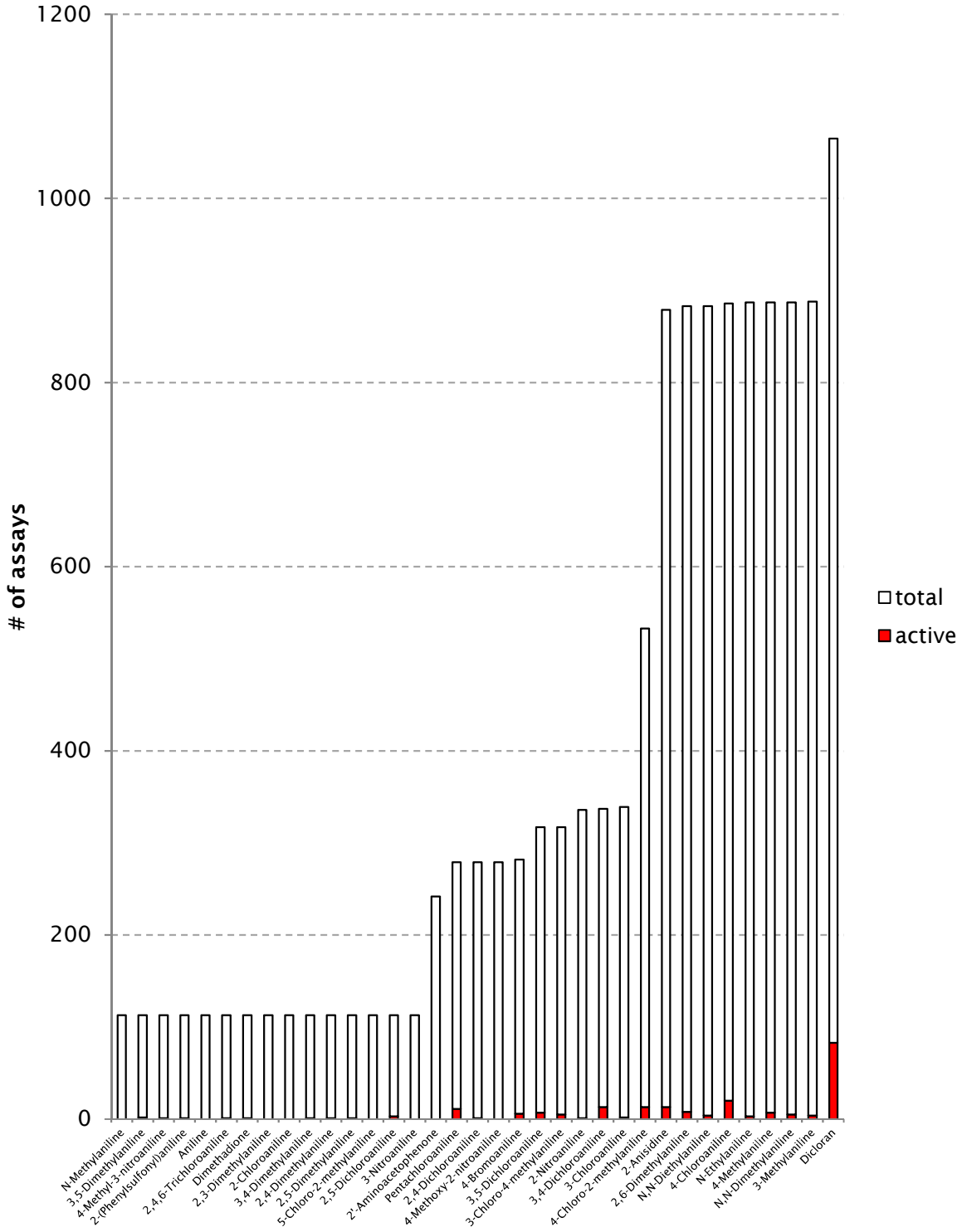
	Name assay	Model	Format	Time point	Readout	Related to mode of action	Positive control(s)
A	TOX21_ESRE_BLA_ratio	Hela cells	1536 well plate	24 hr	Quantification expression reporter gene (fluorescence)	Endoplasmatic reticulum stress	17-N-Allylamino-17-demethoxygeldanamycin (17-AAG)
B	CEETOX_H295R_OHPROG_dn	H295R cells	96 well plate	48 hr	Quantification 17α-hydroxyprogesterone (HPLC-MS-MS)	Disruption of steroidogenesis	Prochloraz, Forskolin
C	ATG_PXRE_CIS_up	HepG2 cells	24 well plate	24 hr	Quantification expression reporter gene (RT-PCR)	Activation of enzymes (e.g. CYP3A4)	Rifampicin
D	TOX21_ARE_BLA_agonist_ratio	HepG2 cells	1536 well plate	24 hr	Quantification expression reporter gene (fluorescence)	Oxidative or electrophilic stress response	ß-Naphtoflavone
E	CEETOX_H295R_TESTO_dn	H295R cells	96 well plate	48 hr	Quantification testosterone (HPLC-MS-MS)	Disruption of steroidogenesis	Prochloraz, Forskolin
F	CEETOX_H295R_ANDR_dn	H295R cells	96 well plate	48 hr	Quantification androstenedione (HPLC-MS-MS)	Disruption of steroidogenesis	Prochloraz, Forskolin
G	CEETOX_H295R_11DCORT_dn	H295R cells	96 well plate	48 hr	Quantification 11-deoxycortisol (HPLC-MS-MS)	Disruption of steroidogenesis	Prochloraz, Forskolin
H	ATG_RXRb_TRANS_up	HepG2 cells	24 well plate	24 hr	Quantification expression reporter gene (RT-PCR)	Not defined	None
I	BSK_3C_Proliferation_down	Umbilical vein endothelial cells	96 well plate	24 hr	Quantification protein content (absorbance)	General cell toxicity	Colchicine
J	TOX21_ERa_BLA_Agonist_ratio	HEK293T cells	1536 well plate	24 hr	Quantification expression reporter gene (fluorescence)	Estrogenic activity	17ß-Estradiol

Supplementary Table 8. Background information on the 10 selected ToxCast/Tox21 bioassays for (C)Ps.

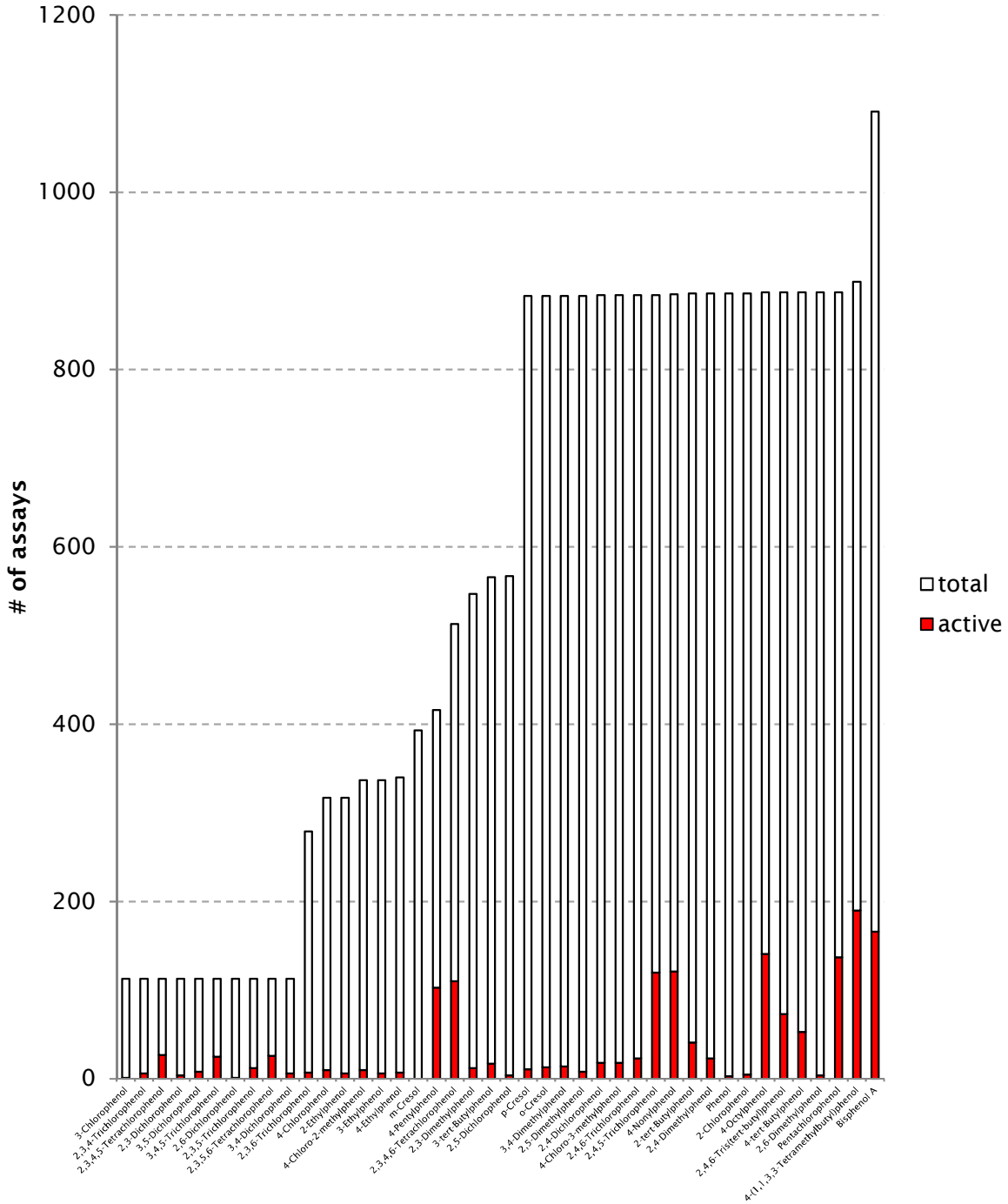
	Name assay	Model	Format	Time point	Readout	Related to mode of action	Positive control(s)
A	TOX21_MMP_ratio_down	HepG2 cells	1536 well plate	24 hr	Mitochondrial membrane potential as determined with fluorescent dye.	Mitochondrial toxicity	Carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP)
B	NHEERL_ZF_144hpf_TERATO SCORE_up	Zebrafish embryos	96 well plate	144 hr	Image-based assessment of morphology	Adverse effects on development	None
C	ATG_ERa_TRANS_up	HepG2 cells	24 well plate	24 hr	Quantification expression reporter gene (RT-PCR)	Estrogenic activity	17ß-Estradiol
D	ATG_NRF2_ARE_CIS_up	HepG2 cells	24 well plate	24 hr	Quantification expression reporter gene (RT-PCR)	Oxidative or electrophilic stress response	4-Hydroxynonenal
E	ATG_PXRE_CIS_up	HepG2 cells	24 well plate	24 hr	Quantification expression reporter gene (RT-PCR)	Activation of enzymes (e.g. CYP3A4)	Rifampicin
F	ATG_ERE_CIS_up	HepG2 cells	24 well plate	24 hr	Quantification expression reporter gene (RT-PCR)	Estrogenic activity	17ß-Estradiol
G	OT_ER_ERbERb_0480	HEK293T	384 well plate	8 hr	Protein-fragment complementation technology (ERß-ERß) (fluorescence)	Estrogenic activity	17ß-Estradiol
H	OT_ER_ERaERb_0480	HEK293T	384 well plate	8 hr	Protein-fragment complementation technology (ERα-ERß) (fluorescence)	Estrogenic activity	17ß-Estradiol
I	BSK_LPS_PGE2_down	Umbilical vein endothelial cells	96 well plate	24 hr	Quantification PGE2 protein expression (ELISA)	Immunosuppression	Colchicine
J	TOX21_ARE_BLA_agonist_ratio	HepG2 cells	1536 well plate	24 hr	Quantification expression reporter gene (fluorescence)	Oxidative or electrophilic stress response	ß-Naphtoflavone



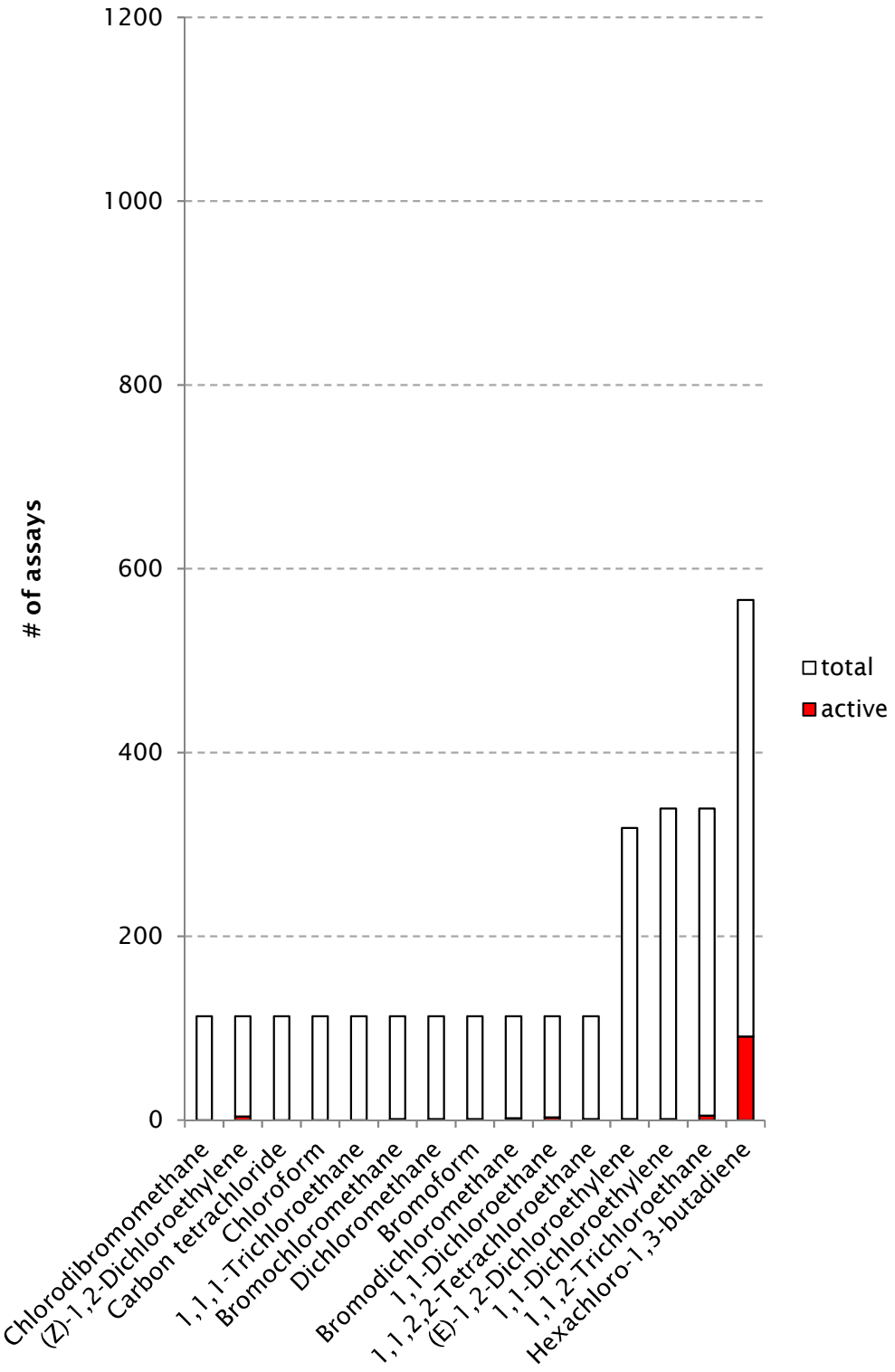
Supplementary Figure 1. Overview of number of bioassays in which PAHs have been tested (total column) and in which they are active (red column).



Supplementary Figure 2. Overview of number of bioassays in which AAs which are measured as target by Vitens drinking water company have been tested (total column) and in which they are active (red column).



Supplementary Figure 3. Overview of number of bioassays in which (C)Ps which are measured as target by Vitens drinking water company have been tested (total column) and in which they are active (red column).



Supplementary Figure 4. Overview of number of bioassays in which HAHs which are measured as target by Vitens drinking water company have been tested (total column) and in which they are active (red column).