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Short Communication

Exploration of ToxCast/Tox21 bioassays as candidate bioanalytical tools for measuring groups of chemicals in water

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HIGHLIGHTS

- This study aims to identify candidate bioassays for detecting groups of chemicals.
- EPA's ToxCast/Tox21 database was explored for candidate bioassays.
- Bioassays were identified for polycyclic aromatic hydrocarbons and (chloro)phenols.
- Candidate bioassays should be further evaluated for use in water quality monitoring.

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ABSTRACT

The present study explores the ToxCast/Tox21 database to select candidate bioassays as bioanalytical tools for measuring groups of chemicals in water. To this aim, the ToxCast/Tox21 database was explored for bioassays that detect polycyclic aromatic hydrocarbons (PAHs), aromatic amines (AAs), (chloro)phenols ((C)Ps) and halogenated aliphatic hydrocarbons (HAliHs), which are included in the European and/or Dutch Drinking Water Directives. Based on the analysis of the availability and performance of bioassays included in the database, we concluded that several bioassays are suitable as bioanalytical tools for assessing the presence of PAHs and (C)Ps in drinking water sources. No bioassays were identified for AAs and HAliHs, due to the limited activity of these chemicals and/or the limited amount of data on these chemicals in the database. A series of bioassays was selected that measure molecular or cellular effects that are covered by bioassays currently in use for chemical water quality monitoring. Interestingly, also bioassays were selected that represent molecular or cellular effects that are not covered by bioassays currently applied. The usefulness of these newly identified bioassays as bioanalytical tools should be further evaluated in follow-up studies. Altogether, this study shows how exploration of the ToxCast/Tox21 database provides a series of candidate bioassays as bioanalytical tools for measuring groups of chemicals in water. This assessment can be performed for any group of chemicals of interest (if represented in the database), and may provide candidate bioassays that can be used to complement the currently applied bioassays for chemical water quality assessment.

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, also indicator parameters for groups of chemicals are included, such as aromatic amines (AAs), (chloro)phenols ((C)Ps) and halogenated aliphatic hydrocarbons (HAliHs) (Drinkwaterbesluit). In the standards for groups of chemicals the maximum summed concentration allowed in drinking water is defined, e.g. 0.1 μg/L for a selection

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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 groups of chemicals in these monitoring programs, but these only cover a pre-selected set of chemicals. With this approach, other chemicals within the same groups 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, 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., under review) and legal embedding of the bioassays in the EU DWD and Water Framework Directive (WFD, Brack et al., 2017). Several *in vitro* bioassays, covering diverse chemical-induced molecular or cellular responses, are currently applied in chemical water quality assessment. Bioassays that are currently applied in water quality monitoring have been selected based on knowledge on chemical-induced molecular or cellular responses of chemicals relevant for water, their related costs and their ease-of-use (Schriks et al., 2015). Although several of these bioassays have already proven their usefulness in chemical water quality assessment, other useful bioassays, measuring chemical-induced molecular or cellular responses that are not yet covered by the currently applied bioassays, may be available.

The present study investigates whether the high throughput EPA ToxCast and Tox21 databases (https://actor.epa.gov/dashboard) can be used to explore candidate bioassays to measure defined groups of chemicals in water. To this aim, *in vitro* data on all bioassays in the database were explored for groups of chemicals that are included in the European and/or Dutch DWD. The groups of chemicals 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 HAliHs, for which indicator parameters have been defined in the Dutch DWD.

2. Methods

We first assessed which chemicals that are measured by Vitens, a Dutch drinking water company, to monitor the chemical groups PAHs, AAs, (C)Ps and HAliHs (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 chemical group of interest have been tested and whether the tested chemicals are active (i.e. effect concentrations are available). We first made for each chemical group a preselection of 20 possibly relevant bioassays based on the highest number of active chemicals of the group. The performance of these preselected bioassays was subsequently evaluated in more detail on i) inclusiveness, ii) responsiveness and iii) specificity for the defined chemical group. These parameters are considered of equal importance and represent: i) the number of active chemicals of the chemical group as a percentage of the number of chemicals that were considered for that group and ii) the number of active chemicals of the group as a percentage of the number of the chemicals of that group that has been tested, and iii) the number of the active chemicals of the group as a percentage of all active chemicals in that bioassay as reported in the ToxCast/Tox21 database. Bioassays in which many of the chemicals of interest have been tested score high on inclusiveness if these chemicals are active. If few of the chemicals of interest have been tested in a bioassay, the bioassay scores low on inclusiveness, even when the tested chemicals are active. Regarding responsiveness, a bioassay scores high if (most of) the tested chemicals are positive, even when few chemicals have been tested in that bioassay. A bioassay scores high for specificity if besides the chemicals of interest, few other chemicals in the database give a response in that bioassay. This can be useful if a bioassay is used to detect chemicals belonging to a specific (defined) group of chemicals.

Further, it was assessed which average relative enrichment factor (REF) would be required to obtain a bioassay response equal to the activity concentration of the cutoff (ACC) when chemicals would be present in water at concentrations equal to the related guideline value or indicator parameter as defined in the DWD (0.1 μg/L for PAHs, 1.0 μg/L for AAs, (C)Ps and HAliHs). The cutoff is defined as the baseline response +3 times the baseline median absolute deviation (BMAD: noise around the baseline). This information on sensitivity was not used to rank bioassays, since the sensitivity of bioassays is chemical-specific and ACC values for different chemicals within a defined group can differ orders of magnitude in a single bioassay. However, bioassays for which an average active REF higher than 100 000 is required were excluded for further analysis, because their sensitivity was regarded to be too low. Based on these analyses, for each chemical group, 10 bioassays were selected from the 20 preselected bioassays as having the highest potential to be used in water quality monitoring for these chemical groups.

3. Results

For the PAHs, 5 individual PAHs have been defined in the European DWD and 11 in the Dutch DWD, whereas for the Dutch indicator parameters for AAs, (C)Ps and HAliHs individual chemicals have not been defined. In practice, drinking water companies have implemented their own selection of target chemicals that cover these chemical groups. The ToxCast/Tox21 database contains data for the 11 PAHs, 35 of the AAs, 42 of the (C)Ps and 15 of the HAliHs (being 100%, 76%, 91% and 94% of the chemical group members routinely monitored by Vitens) (Supplementary Tables 1–4 and Supplementary Figs. 1–4). Fig. 1 shows for each chemical group the number of ToxCast/Tox21 bioassays as a function of the percentage of chemicals included in the chemical group that has been tested and of the percentage of tested chemicals included in the chemical group that is active. In general, the number of bioassays in which the majority of chemicals included in these chemical groups is active is low, especially for the AAs and HAliHs. Besides, HAliHs have been tested in relatively few bioassays. Therefore, AAs and HAliHs are not further explored in the present study. For both 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). No bioassay endpoints were included that cannot be considered as standalone readouts (e.g. endpoints used for background signal correction). From these priority lists, bioassays were removed in which the

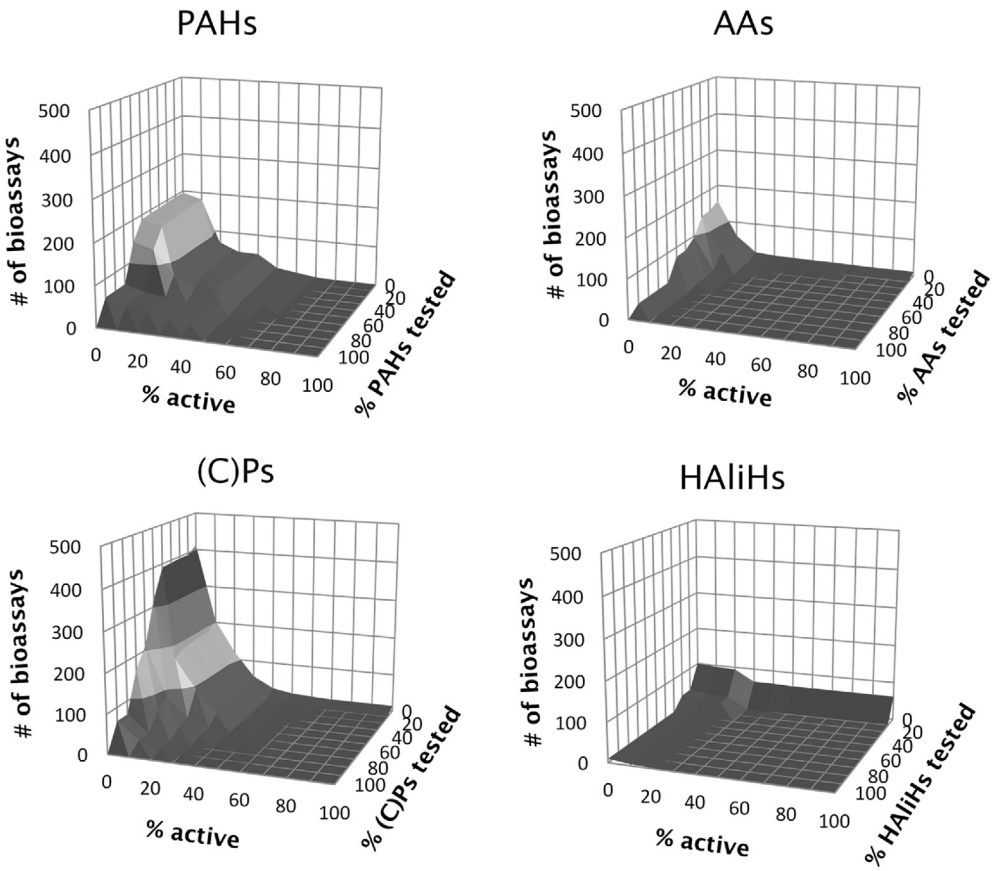

Fig. 1. Activity of selected groups of polycyclic aromatic hydrocarbons (PAHs), aromatic amines (AAs), (chloro)phenols (C)Ps and halogenated aliphatic hydrocarbons (HAliHs) in ToxCast/Tox21 bioassays. The graphs present the number of bioassays as a function of the percentage of chemicals of the chemical group that has been tested and of the percentage of the tested chemicals that is active.

average REF required is higher than 100 000 (Supplementary Table 5, bioassays 9–12, 16). From the remaining bioassays, the 10 with the highest scores for the sum of the three evaluation criteria were selected.

3.1. PAHs

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 11 PAHs. Table 1 summarizes the performance of the selected bioassays based on i) inclusiveness, ii) responsiveness and iii) specificity for the chemical group PAHs and presents the average active concentrations. The scores on inclusiveness range between 36% and 64% 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). On the other hand, regarding sensitivity of the bioassays, the TOX21_ARE_BLA_agonist_ratio bioassay (Table 1, bioassay D) and the TOX21_AhR_LUC_Agonist bioassay (Table 1, bioassay E) are more promising, given the lower average REFs required for these bioassays to obtain a response when the PAHs are present in water at a concentration that equals the guideline value of 0.10 μg/L.

The selected bioassays include bioassays that represent diverse molecular or cellular effects, including endoplasmatic reticulum stress (A), oxidative or electrophilic stress (D), mitochondrial membrane potential decrease (H), induction of xenobiotic metabolism genes (C, E) and endocrine modulation (estrogen receptor activation (F, G) and inhibition of steroidogenesis (B, I, J)). Some of these molecular or cellular effects are covered by bioassays that are currently already applied for chemical water quality monitoring (C, D, E, F, G) (Tang et al., 2013; Escher et al., 2014; Leusch et al., 2014a; b; Di Paolo et al., 2016; Leusch et al., 2017; Neale et al., 2017; Altenburger et al., 2018), whereas others are not (A, B, H, I, J).

3.2. (C)Ps

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 chemical group (C)Ps and presents the average active concentrations. The scores on inclusiveness ranges between 26% and 62% and the scores on responsiveness between

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 activity concentration at the cutoff (ACC; mg/L) is given. 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 (mg/L) of the active PAHs) is presented as well as the average REF required for the active PAHs when present at a concentration of 0.1 μg/L (guideline value for group of PAHs) to reach the ACC.

Name	CAS	A	B	C	D	E	F	G	H	I	J
Anthracene	120-12-7	8.3						11			
Benz(a)anthracene	56-55-3	13	1.7	1.9	0.77	0.22	10	4.5	5.9	1.5	1.5
Benzo(a)pyrene	50-32-8	0.23			0.14	0.21	0.54	11	6.1		
Benzo(b)fluoranthene	205-99-2	3.5	1.9		0.11	0.049	7.8	13	7.3		
Benzo(g,h,i)perylene	191-24-2	0.40			1.0						
Benzo(k)fluoranthene	207-08-9	0.040			0.035	0.0014	0.081				
Chrysene	218-01-9				2.4	0.71					
Fluoranthene	206-44-0	10	2.6	4.7			11	4.9	14	5.1	6.1
Indeno(1,2,3-cd)pyrene	193-39-5			0.73							
Phenanthrene	85-01-8		6.8	9.8						8.5	11
Pyrene	129-00-0		3.4	1.7					14	7.0	8.1
# of positive PAHs		7	5	5	6	5	5	5	5	4	4
Evaluation citeria											
i) inclusiveness (%)		64	45	45	55	45	45	45	45	36	36
ii) responsiveness (%)		70	71	71	60	50	50	50	50	57	57
iii) specificity (%)		2.0	3.2	0.3	0.4	0.6	1.0	0.4	0.5	2.6	2.1
Sum evaluation criteria i-iii		136	120	117	115	96	96	96	96	96	96
average active concentration (mg/L)		5.1	3.3	3.8	0.74	0.24	5.9	8.7	9.6	5.5	6.7
average REF required		51002	32835	37758	7446	2387	58941	87001	95667	55374	67301

A. TOX21_ESRE_BLA_ratio
B. CEETOX_H295R_OHPROG_dn
C. ATG_PXRE_CIS_up
D. TOX21_ARE_BLA_agonist_ratio
E. TOX21_AhR_LUC_Agonist
F. TOX21_ERa_BLA_Agonist_ratio
G. TOX21_ERa_LUC_BG1_Agonist
H. TOX21_MMP_ratio_down
I. CEETOX_H295R_TESTO_dn
J. CEETOX_H295R_ANDR_dn

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 NHEERL_ZF_144hpf_TERATOSCORE_up bioassay (Table 2, bioassay B) and the ATG_ERa_TRANS_up bioassay (Table 2, bioassay C). On the other hand, regarding sensitivity of the bioassays, the BSK_LPS_PGE2_down bioassay (Table 2, bioassay I) and the ATG_PXRE_CIS_up bioassay (Table 2, bioassay E) are more promising, given the lower average REFs required for these bioassays to obtain a response when the (C)Ps are present in water at a concentration that equals the indicator parameter value of 1.0 μg/L.

The selected bioassays include bioassays that represent diverse molecular or cellular effects, including oxidative or electrophilic stress (D, J), mitochondrial effects (A), activation of nuclear receptors that regulate expression of xenobiotic metabolism genes (E), endocrine modulation (estrogen receptor activation (C, F, G, H)) and immunomodulation (inhibition of lipopolysaccharide (LPS)-induced prostaglandin (PGE2) expression (I)). Further, one bioassay was selected that assesses the effects of chemicals on the development of zebrafish embryos (B). Some of these molecular or cellular effects are covered by bioassays that are currently already applied for chemical water quality monitoring (C, D, E, F, G, H, J) (Tang et al., 2013; Escher et al., 2014; Leusch et al., 2014a; b; Di Paolo et al., 2016; Leusch et al., 2017; Neale et al., 2017; Altenburger et al., 2018), whereas others are not (A, I). Further, zebrafish-based bioassays (B) are currently also already applied in chemical water quality monitoring (Thellmann et al., 2015, 2017; Babić et al., 2017).

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 activity concentration at the cutoff (ACC; mg/L) is given. 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 (mg/L) of the active (C)Ps) is presented, as well as the average REF required for the active (C)Ps when present at a concentration of 1 μg/L (guideline value for group of (C)Ps) to reach the ACC.

(C)P	CAS	A	B	C	D	E	F	G	H	I	J
Bisphenol A	80-05-7	3.3	11	0.0076	3.6	0.17	0.011	0.023	0.067	5.2	4.2
2-Ethylphenol	90-00-6				7.0	0.58					
3-Ethylphenol	620-17-7					1.4					11
4-Ethylphenol	123-07-9			6.1			22		5.5		
4-Chloro-2-methylphenol	1570-64-5	9.4		7.7	8.0		8.0	8.0	5.1		
4-Chloro-3-methylphenol	59-50-7	4.7	8.8	3.8			5.2	2.2	3.3		
2-Chlorophenol	95-57-8				17						
3-Chlorophenol	108-43-0	8.9									
4-Chlorophenol	106-48-9			6.6			19	5.4	4.4		
m-Cresol	108-39-4										
o-Cresol	95-48-7									1.8	
p-Cresol	106-44-5									0.72	
2,3-Dichlorophenol	576-24-9	8.4									
2,4-Dichlorophenol	120-83-2	4.6	6.2	9.8	4.1	13	9.8	11	7.3		
2,5-Dichlorophenol	583-78-8	5.8									
2,6-Dichlorophenol	87-65-0										
3,4-Dichlorophenol	95-77-2	1.8									7.1
3,5-Dichlorophenol	591-35-5	1.9									
2,3-Dimethylphenol	526-75-0			3.4		3.7	0.003			5.2	
2,4-Dimethylphenol	105-67-9			24						0.32	
2,5-Dimethylphenol	95-87-4									1.1	
2,6-Dimethylphenol	576-26-1			8.5						0.58	
3,4-Dimethylphenol	95-65-8			8.6	13		18			0.30	
4-Nonylphenol	104-40-5	2.5	9.6	0.91	2.1	2.8	0.32	2.0	1.7	0.66	11
4-Octylphenol	1806-26-4	2.3	0.83	0.34	1.8	2.0	0.40	0.93	0.88	0.61	16
Pentachlorophenol	87-86-5	0.51	0.14		1.3	1.3					3.0
4-Pentylphenol	14938-35-3			0.15	1.3	2.9	0.14			0.82	
Phenol	108-95-2										
2,3,4,5-Tetrachlorophenol	4901-51-3	1.6									3.2
2,3,4,6-Tetrachlorophenol	58-90-2	3.4			8.8	5.6					6.0
2,3,5,6-Tetrachlorophenol	935-95-5	2.2									4.3
4-(1,1,3,3-Tetramethylbutyl)phenol	140-66-9	1.2	0.74	0.29	1.5	0.24	0.10	0.059	0.083		5.4
2-tert-Butylphenol	88-18-6	9.9		2.2	7.2			7.4			
3-tert-Butylphenol	585-34-2	9.1		1.7		0.97	10	3.6	3.1		
4-tert-Butylphenol	98-54-4	7.2	5.8	0.13	12	2.3	0.24	1.2	1.8	0.56	
2,3,4-Trichlorophenol	15950-66-0	1.5									11
2,3,5-Trichlorophenol	933-78-8	1.7									9.0
2,3,6-Trichlorophenol	933-75-5	9.9				7.7					16
2,4,5-Trichlorophenol	95-95-4	1.1	0.81	3.1	4.6	3.4	4.4	9.0	9.0		9.5
2,4,6-Trichlorophenol	88-06-2	11	0.75	14	13	15					7.9
3,4,5-Trichlorophenol	609-19-8	1.0									2.3
2,4,6-Tris(tert-butyl)phenol	732-26-3	11	6.0		16	0.62	1.5	11	14		
# 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 concentration (mg/L)		4.8	4.6	5.3	7.3	3.7	6.3	4.8	4.3	1.5	7.9
average REF required		4848	4628	5342	6864	3532	6268	4750	4325	1495	7933

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
J. TOX21_ARE_BLA_agonist_ratio

4. Discussion

The aim of the present study was to assess whether exploration of the ToxCast/Tox21 database can provide candidate bioassays that can be used in water quality assessment to determine the presence of groups of chemicals, using PAHs, AAs, (C)Ps and HAliHs as examples. Our analysis shows that bioassays included in the ToxCast/Tox21 database can be used as bioanalytical tools to assess the presence of PAHs and (C)Ps, but that insufficient data are available to evaluate the use of bioanalytical tools to measure AAs and HAliHs.

In our analysis, we did not use prior knowledge on the mode of action of chemicals for the selection of bioassays, but used an hypothesis free approach. Using our approach we (pre)selected candidate bioassays that cover molecular or cellular effects that are already represented by various bioassays currently applied in chemical water quality monitoring, but also bioassays that cover cellular or molecular effects that are, as far as we know, not yet represented by currently applied bioassays. Already represented molecular or cellular effects include bioassays that measure oxidative and electrophilic stress responses (related to activation of the Nrf2-pathway (ATG_NRF2_ARE_CIS_up and TOX21_ARE_-BLA_agonist_ratio)), bioassays that measure molecular processes related to the increased expression of cytochrome P450 genes ('xenobiotic metabolism'; ATG_PXRE_CIS_up and TOX21_-AhR_LUC_Agonist), and bioassays that measure activation of the estrogen receptor ('endocrine modulation'; ATG_ERa_TRANS_up, ATG_ERE_CIS_up, OT_ER_ERaERb_0480, OT_ER_ERbERb_0480, TOX21_ERa_LUC_BG1_Agonist and TOX21_ERa_BLA_Agonist_ratio) (Tang et al., 2013; Escher et al., 2014; Leusch et al., 2014a; b; Di Paolo et al., 2016; Leusch et al., 2017; Neale et al., 2017; Altenburger et al., 2018). Interestingly, our analysis also directed to bioassays that cover cellular or molecular effects that are, as far as we know, not represented by bioassays that are commonly applied in chemical water quality monitoring studies. These include chemical-induced endoplasmatic reticulum stress (TOX21_ESRE_-BLA_ratio; by PAHs), inhibition of steroidogenesis (CEETOX_H295R_OHPROG_dn, CEETOX_H295R_TESTO_dn and CEETOX_H295R_ANDR_dn; by PAHs), decrease of mitochondrial membrane potential (TOX21_MMP_ratio_down; by PAHs and (C)Ps), and immunomodulation (BSK_LPS_PGE2_down; by (C)Ps). These newly identified bioassays may be interesting candidate bioassays to further evaluate for application in water quality assessment and may be important additions to the currently applied set of bioassays.

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. This indicates that a combination of bioassays is required to detect as many of the PAHs and (C)Ps as possible. Incomplete detection of all compounds in a group is only problematic if the missing chemicals are present in the monitored waters. Interestingly, the PXRE_CIS_up bioassay, the TOX21_ARE_BLA_agonist_ratio bioassay and the TOX21_MMP_ratio_down 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. Indeed, the molecular or cellular effects represented by these bioassays rather detect non-specific effects, being activation of oxidative/electrophilic stress response, PXR activation, and decrease of mitochondrial membrane potential, indicating that they will detect chemicals of diverse nature.

It is important to note that chemicals 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 currently used as guideline values or indicator parameters for chemical groups (i.e. 0.10 μg/L for PAHs and 1.0 μg/L for (C)Ps). Therefore, it is clear that bioassays cannot be used as a direct replacement of chemical analyses to determine the total concentration of chemical groups in water. However, since chemical analyses only cover a defined set of (known) target chemicals, bioassays serve as an important complementary tool to detect unknown or undetected (related) bioactive chemicals. The present study has identified candidate bioassays that can be used in such an approach to detect PAHs and (C)Ps that are not detected using targeted analyses. To further analyse the potential of the selected candidate bioassays, studies are required that further assess their application, taking into account criteria that are of importance for the application of bioassays for water quality monitoring, such as ease of use and costs, which have been defined in the EU FP7 project DEMEAU (Schriks et al., 2015).

The present study shows that the specificity of the selected candidate bioassays is low for both PAHs and (C)Ps. Therefore, they are not suitable to specifically measure these groups of chemicals, since other chemicals present in water may also provoke a response. Not surprisingly, the bioassays that measure oxidative and electrophilic stress responses (Table 1, bioassay D; Table 2, bioassays D and J) and activation of nuclear receptors that regulate CYP450 expression (Table 1, bioassays C and E; Table 2, bioassay E) have the lowest scores for specificity. However, also bioassays that measure more specific effects score rather low on specificity. A low specificity is not necessarily a problem, especially if the chemical-induced bioassay response can be related to known adverse effects of (the majority) of the chemicals that elicit the response. In that case bioassays that detect many chemicals (instead of only those belonging to a specific chemical group) can be considered to be an advantage. Importantly, for the interpretation of responses obtained in bioassays, health-based effect-based trigger (EBT) values need to be defined that are used to evaluate the health relevance of detected bioassay responses (Brand et al., 2013; Escher et al., 2015, 2018).

In the context of the objective of the present study to assess whether bioassays can be used to detect PAHs and (C)Ps at concentrations that are defined as guideline value (PAHs: 0.1 μg/L) or indicator parameter ((C)Ps: 1.0 μg/L), it was assessed which average REFs are required to obtain a bioassay response at these concentrations. The results show that the required REFs are higher than 1500, and are therefore considered unrealistically high. This indicates that the sensitivity of the bioassays evaluated in this study does not suffice to detect chemicals present at guideline value concentrations. 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). On the other hand, since guideline values and indicator parameters are not necessarily health-based, and if bioassay responses are to be used to determine whether adverse health effects can be expected, the sensitivity of the bioassays should be evaluated using health based EBT values, which are based on information on safe concentrations of chemicals in water (Brand et al., 2013).

The present study shows that the ToxCast/Tox21 bioassays hardly detect AAs and HAliHs. This information adds insights into which chemicals present in water cause bioassay responses and which do not, which is required to apply bioanalytical tools together with chemical analytical tools in chemical water quality assessment. This information can also be used to direct bioassay research to the development of bioassays for these (groups of)

chemicals. Basic knowledge on modes of action underlying the toxicity of AAs and HAliHs may provide a starting point to develop bioassays that detect these chemicals. As such, the development of the adverse outcome pathway (AOP)-concept, in which the consecutive biological events from the first interaction of the chemical with the biological system (molecular initiating event) to the toxic effect (adverse outcome) are described, is of great value, as it can be used to align bioassays to the biological events that play a role in the toxicity of the chemicals (Ankley et al., 2010; Vinken, 2013).

It must be noted that the ToxCast/Tox21 database is not a homogenous set of data in which all chemicals have been tested in all bioassays. It is therefore possible that appropriate bioassays may not have been selected because of the lack of data for the chemicals we focused on. Although the scores on inclusiveness, responsiveness and specificity help to select candidate bioassays in the present study, they should be interpreted with care, as they have been derived from the non-complete dataset. If new data become available for these chemicals, the analysis should be repeated, and other scores for the evaluation criteria may be obtained possibly resulting in a different selection of candidate bioassays.

In conclusion, the present study shows an approach of how the ToxCast/Tox21 database can be used to select candidate bioassays to detect groups of chemicals in drinking water. It revealed candidate bioassays for both PAHs and (C)Ps that represent molecular or cellular effects that are covered by bioassays that are currently used for chemical water quality assessment, as well as candidate bioassays that represent molecular or cellular effects that are not covered by the currently applied bioassays. It also showed that the ToxCast/Tox21 bioassays hardly detect AAs and HAliHs. The approach described in the present study can be used as a first step to select candidate bioassays for each group of chemicals of interest that is adequately represented in the ToxCast/Tox21 database.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2018.06.056.

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