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Assessment of ecotoxicity and total volatile organic compound (TVOC) emissions from food and children's toy products

Natalia Szczepańska[1], Mariusz Marć[*1,3], Błażej Kudłak[1],Vasil Simeonov[2], Stefan Tsakovski[2], Jacek Namieśnik[1]

[1]Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, 11/12 Narutowicza Str., 80-233 Gdańsk, Poland

[2]Chair of Analytical Chemistry, Faculty of Chemistry and Pharmacy, University of Sofia "St. Kl. Okhridski", 1, J. Bourchier Blvd., 1164 Sofia, Bulgaria

[3]Department of Analytical and Ecological Chemistry, Faculty of Chemistry, Opole University, pl. Kopernika 11a, 45-040 Opole, Poland

*Corresponding Author: marmarc@pg.edu.pl

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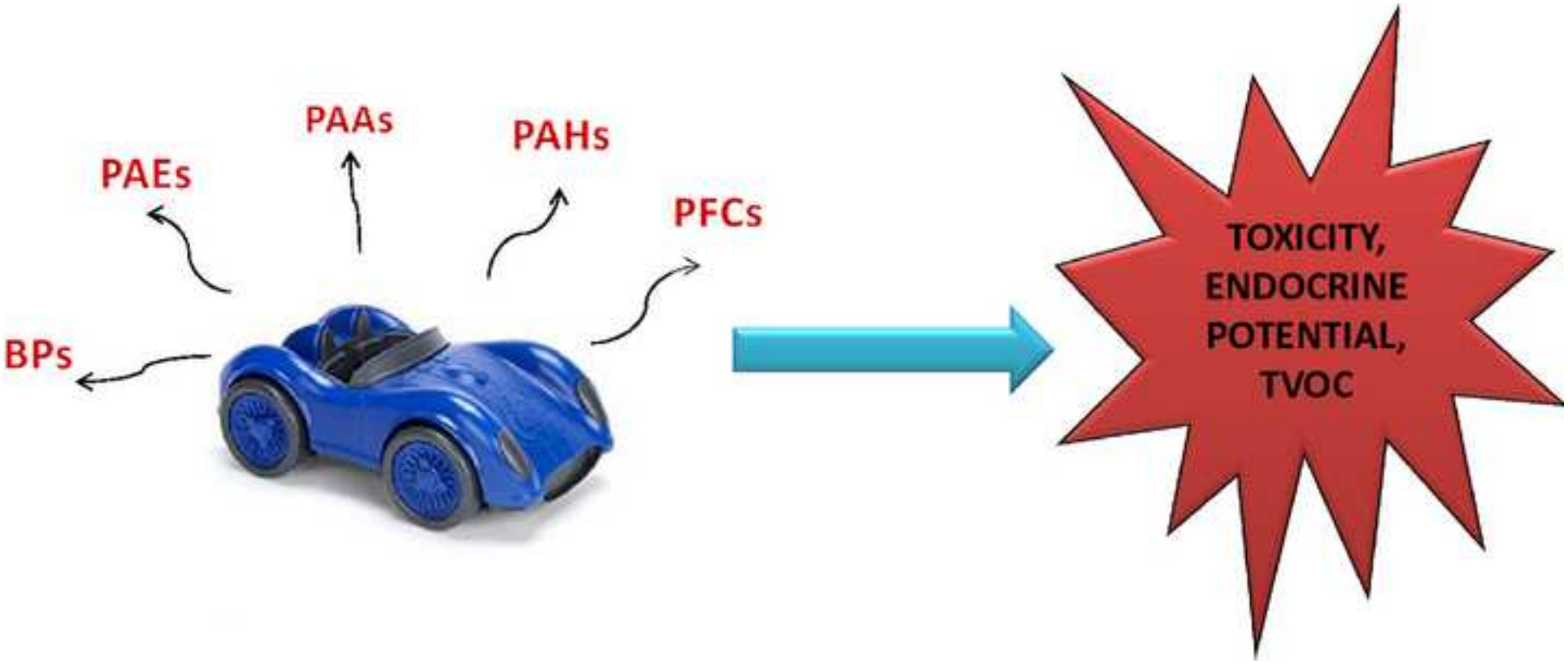
Graphical Abstract



Highlights

- The assessing studies of total volatile organic compounds emissions from plastic materials were performed;
- The impact of time on toxicity of extracts of product devoted for children was investigated;
- The endocrine potential of extracts of investigated objects was assessed;
- The correlation between total volatile organic compounds emission and toxicity of children's products was estimated;
- The mathematical assessment approach was applied in the field of ecotoxicity modeling studies

Abstract

The development of new methods for identifying a broad spectrum of analytes, as well as highly selective tools to provide the most accurate information regarding the processes and relationships in the world, has been an area of interest for researchers for many years. The information obtained with these tools provides valuable data to complement existing knowledge but, above all, to identify and determine previously unknown hazards. Recently, attention has been paid to the migration of xenobiotics from the surfaces of various everyday objects and the resulting impacts on human health. Since children are among those most vulnerable to health consequences, one of the main subjects of interest is the migration of low-molecular-weight compounds from toys and products intended for children. This migration has become a stimulus for research aimed at determining the degree of release of compounds from popular commercially available chocolate/toy sets. One of main objectives of this research was to determine the impact of time on the ecotoxicity (with *Vibrio fischeri* bioluminescent bacteria) of extracts of products intended for children and to assess the correlation with total volatile organic compound emissions using basic chemometric methods. The studies on endocrine potential (with XenoScreen YES/YAS) of the extracts and showed that compounds released from the studied objects (including packaging foils, plastic capsules storing toys, most of toys studied and all chocolate samples) exhibit mostly androgenic antagonistic behavior while using artificial saliva as extraction medium increased the impact observed. The impact of time in most cases was positive one and increased with prolonging extraction time.

The small-scale stationary environmental test chambers - μ-CTE™ 250 system was employed to perform the studies aimed at determining the profile of total volatile organic compounds (TVOCs) emissions. Due to this it was possible to state that objects from which the greatest amounts of contaminants are released are plastic containers (with emission rate falling down from 3273 to 2280 ng/g of material at 6 h of conditioning in elevated temperature).

Keywords: TVOC; ecotoxicity; endocrine potential; chemometric methods; children's toys

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1. Introduction

For parents around the world, the health and wellbeing of their children is an issue of paramount importance. Still, despite numerous legislative and procedural difficulties for producers of substandard toy and food producers, several accidents occurred that forced the scientific community to search for new ways to respond to this threat. Promisingly, research is being conducted to combine biological, instrumental and chemometric studies to assure holistic assessment of the quality of products intended for children and determine the impact on human well-being.

To rapidly obtain screening data about the estimated amount of volatile organic compounds (VOCs) released from the surfaces of various types of products into indoor air, research is carried out to assess the values of total volatile organic compounds (TVOCs) in a defined medium. According to published information, TVOC parameter is determine as the sum (total amount) of wide spectrum of organic compounds eluting between the defined analytical window - retention times from n-hexane to n-hexadecane on an appropriate gas chromatography capillary column (non-polar or slightly polar stationary phases) employing flame ionization detection (GC-FID) and quantified as toluene equivalents (ECA, 1997; Formela et al., 2017, 2016; Ghaffar et al., 2014; Hakkarainen, 2010; Kaykhaii and Linford, 2017; Liu et al., 2012; Massold et al., 2005). Chemical compounds such as aromatic hydrocarbons, aliphatic hydrocarbons, monoterpenes, alcohols, aldehydes and ketones (excluding formaldehyde) have a significant impact on the final value of the TVOC parameter. Depending on the object investigated, the intensity of the impact of a selected group of organic compounds on the final TVOC value varies. Preliminary determination of TVOC values allows researchers to obtain screening information on the quality of studied materials and make preliminary comparisons. In addition, the TVOC parameter consists of chemical compounds defined by the International Agency for Research on Cancer (IARC) as carcinogenic such as benzene (Group 1A), probably carcinogenic (Group 2A), or possibly carcinogenic like styrene or ethylbenzene (Group 2B) (IARC, 2016). Therefore, assessing TVOC values enables researchers to perform preliminary evaluations of the impact of studied objects on human health or the environment.

Without doubt, biological tests are becoming increasingly important in analytical practice. The main advantage of diagnostic tools is their capacity to specify the actual influence of compounds in tested samples on living organisms, taking into account all interactions between them (Wieczerzak et al., 2016). In the literature, there are many examples of studies showing

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that the coexistence of several pollutants leads to more severe adverse effects than predicted based on the toxicity of individual components (Backhaus, 2012; Pose-Juan et al., 2016; Sexton and Hattis, 2007; Silva et al., 2002). In most cases, such interactions were found in compounds such as bisphenol A and related compounds, phthalates, primary aromatic amines, and heavy metals (Abdul-Ghani et al., 2012; Fic et al., 2014; Michałowicz, 2014; Ramirez et al., 2014; Xu et al., 2014). In a study on the individual and combined effects of bisphenol A, dibutyl phthalate, and cadmium on oxidative stress and genotoxicity in HepG 2 cells, synergistic interactions were noted (Li et al., 2017). In another study, a similar harmful effect was observed between nonylphenol and di-N-butyl phthalate (Hu et al., 2014). These compounds are plasticizers, additives and printing inks also commonly used in the production of toys and other products intended for children (Lv et al., 2015; Szczepanska et al., 2016). Considering these findings, it seems necessary to intensify efforts to accurately estimate the degree of compound mobility and the effects of compound interactions on living organisms, particularly for children who are most vulnerable to the threat. Their tendency to get to know the world with their mouths in the early stages of life combined with weaker detoxicating abilities makes contact with xenobiotics a potential contributing factor to serious health consequences (Damstra, 2002; Mercan et al., 2015).

Therefore, the use of bioanalytical methods has become particularly important in research aimed to estimate the degree of xenobiotic migration from object surfaces. It might be observed that there is no information in the literature on the use of such a joint approach in research aimed at estimating the degree of xenobiotic migration from the surface of toys to liquid and gaseous phase. Therefore, the current research will make a significant contribution to knowledge about the degree of compound mobility and the resulting impacts on healthy children.

The main aim of the present study was to determine the impact of time on the ecotoxicity of samples extracted from products intended for children and to evaluate the correlation with TVOC emissions using basic mathematical methods performed on appropriate statistical software. The ecotoxicological tests used were Microtox® and XenoScreen YES/YAS, while TVOC emission rates were evaluated using a small-scale stationary emission chamber system at the samples seasoning stage. The thermal desorption technique (TD) combined with gas chromatography technique (GC) equipped with flame ionization detection (FID) was employed at the stage of liberation and final determination of VOCs. This mathematical approach is based on best-fit function modeling, with ecotoxicity or TVOC concentration as dependent variable *Y* and time as independent variable *X*. As in previous studies on

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ecotoxicity modeling, the most appropriate model seems to be the polynomial fit of the $Y = a_1X^2 + a_2X + b$ type, which accurately describes the experimental data. The weight coefficient $\boldsymbol{a_1}$ (by value and by sign) can be used to interpret the impact of time. Model validation was performed using R^2 values (multiple correlation coefficients). The research protocol presented in the following paper is an important example of interdisciplinary studies, combining aspects of analytical chemistry, environmental chemistry, ecotoxicology and mathematical/chemometric methods. All of these elements should be considered in seeking novel approaches to evaluating the quality of polymer children's toys and assessing the potential impacts on human health.

2. Materials and Methods

2.1. Objects studied

The objects studied were all elements of popular commercially available chocolate/toy sets, presented in Figure 1 for easier visualization. All elements (sealing aluminum foil package, chocolate, plastic package for toy, toy itself) of both the original product and its cheaper equivalent were subjected to both toxicological and TVOC studies in duplicate.

2.2. Extraction studies

Extraction conditions were selected to reflect plausible forms of human contact with the objects of interest. Two liquids were used as extraction media: distilled water and artificial saliva (NaCl 0.53 g/dm^3, KCl 0.33 g/dm^3, $CaCl_2 \cdot 2H_2O$ 0.15 g/dm^3, $K_2HPO_4 \cdot 3H_2O$ 0.76 g/dm^3, $MgCl_2 \cdot 6H_2O$ g/dm^3, K_2CO_3 0.53 g/dm^3, 1 % HCl 0.75 g/dm^3). The experiments were run at room temperature while aliquots of extracts were sampled at 0.5, 1.0, 2.0, 6.0, and 12.0 hours. The objects were placed in glass beakers and flooded with proper extraction media (100 cm^3), sealed with Parafilm® and shaken for proper extraction times. After each period, the aliquots of extracts were sampled and subjected to toxicological studies. Prior to conducting the studies, pH was measured to verify if it was in the range of 6.5 - 7.5. In addition, the chocolate extracts were filtered to increase the clarity of the sample. Detailed information about the chemicals and reagents used in the research were enclosed in the Supplementary Material for Methodology.

2.3. Microtox® studies

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The acute toxicity of extracts was measured with Microtox®. The toxicity was assessed as the inhibition of bioluminescence of the indicator organism (refer to Supplementary Figure 1 for details). Appearance of factors that negatively influence the enzymatic activity of the indicator organism inhibits the oxidation of luciferin, which is manifested as reduced luminescence (Parvez et al., 2006). Because these bioluminescent organisms are highly sensitive to the presence of toxic ingredients, they are used in environmental research to control the degree of toxicity in water, sewage, soils, sludge, ionic liquids, and nanoparticles, among others (Mortimer et al., 2008; Rossetto et al., 2014). To perform calculations it was necessary to calculate the biolouminescence inhibition (BI) [%]. Q-Dixon tests were performed prior to conducting respective calculation with eqs. 1-3:

$$R \quad \text{—} \tag{1}$$

$$\text{———} \tag{2}$$

$$\text{———} \tag{3}$$

where:

I_0 – initial bioluminescene of bacterial suspension,

I_t - bioluminescene of bacterial suspension after 30 min of exposure,

I_{0k} – initial bioluminescene of bacterial suspension exposed to control solution,

I_{tk} - bioluminescene of bacterial suspension after t = 30 min of exposure to control solution,

R_t – correction factor,

G_t – Gamma coefficient,

%SB – bioluminescence inhibition after time *t*.

2.4. XenoScreen YES/YAS studies

The XenoScreen YES/YAS test was used to determine hormonal potential of extracts solutions with respect to oestrogenic, antioestrogenic, androgenic and antiandrogenic activity samples tested as the test uses genetically modified *Saccharomyces cerevisiae* with human oestrogenic and androgenic receptors (hAR) (ref. to Szczepańska et al. 2016 for details)

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(Johnson et al., 2013; Kudłak et al., 2015; Ramirez et al., 2014). The test was performed on the basis of manufacturer's instructions with certain modifications. The general diagram of the XenoScreen YES/YAS procedure used for endocrine potential determinations was shown on the Supplementary Figure 2.

Positive and solvent control were included in which experiment. Final hormone concentration in positive controls ranged from 0.10 to 100 µM. The determinations were repeated three times. For the oestrogenic activity, the growth factor (G) and induction ratio (IR) according to the equations 4 and 5:

(4)

(5)

where:

- $A_{690,S}$ and $A_{570,S}$ is absorbance of samples, respectively, at 690 nm and 570 nm;
- $A_{690,N}$ and $A_{570,N}$ is absorbance of the solvent control at 690 nm and 570 nm, respectively.

It is assumed that the tested sample has agonistic YES/YAS properties if the value of the induction coefficient ≥1.5 (for control/calibrators solutions) and shows antagonistic YES/YAS properties if the value of the induction factor ≤66.7 % of the value obtained for the control/calibrator sample

2.5. TVOC studies and FT-IR analysis of studied products

Estimation of the amount of TVOCs released from the surfaces of studied plastic materials was performed using the four small-scale stationary environmental test chambers - µ-CTE™ 250 system (Markes International, Inc, Gold River, California, USA). Detailed information about the construction, design, operating range, working conditions and fields of application of the µ-CTE™ 250 system is described in previous scientific literature (Marć et al., 2017, 2015; Marć and Zabiegała, 2017; Schripp et al., 2007).

In brief, prior to placing the samples inside the chamber, the small polymeric toys were weighed. Average sample weight was 4.3 ± 1.9 g. Afterwards, selected samples of small polymeric toys were placed inside each of four chambers. Then, the chamber lids were closed and samples were seasoned/conditioned under static conditions (without inert gas flushing) under defined conditions of temperature and time (see Supplementary Table 1). After the

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sample seasoning/conditioning stage, organic compounds emitted from the studied plastic materials into the gaseous phase were purged from the chamber by flushing with inert gas (nitrogen) at the previously defined flow rate. The samples of organic compounds were collected using commercially available cylindrical containers filled with Tenax TA. The TD-GC-FID system was applied to perform liberation and final determination of organic and to assess the TVOC parameter values. More comprehensive information about the working parameters of employed TD-GC-FID system (analytical devices working conditions) is listed in Supplementary Table 1.

In order to obtain the general analytical information about the main type of polymer material that was applied at the manufacturing process of studied brand and non-brand products (toys and capsules), the Fourier transform infrared spectroscopy (FT-IR) analysis was performed. The Thermo Scientific Nicolet 6700^{TM} (USA) spectrometer equipped with ATR accessory with diamond crystal (smart iTR) was employed, to carried out the FT-IR analysis of studied samples. The measurements were conducted with 2 cm^{-1} resolution, in the range from 4000 to 650 cm^{-1}. The OMIC ver. 8.0 was used as the main software to analyze the obtained FT-IR results. Detailed information about the FT-IR analysis of plastic materials might be found elsewhere (Marć et al., 205, Formela et al., 2016, Formela at al., 2017).

2.6. Quality assurance/quality control

For quality assurance, the following parameters were set according to manufacturer's guidelines: for Microtox® I_0 of bacterial suspension >70 U and $CaSO_4$ was used as positive control of bacterial response (as well as $K_2Cr_2O_7$ and 3,5-dichlorophenol routinely considered as positive control/reference solutions) while in case of studies with XenoScreen YES/YAS, the OD_{690} of yeast cultures should be >0.3. In all cases, these requirements were fulfilled.

The assessed values of TVOC emissions were corrected based on the value of the blank/zero sample, which was evaluated for the complete protocol under the conditions used in the analysis of the selected polymeric samples. The analytes recovery from the applied sorption medium - Tenax TA, was performed taking into account the studies in which a well-defined amount of the internal standard solution – toluene d_8, was injected directly onto the previously conditioned and purified sorbent. Next, the Tenax TA stainless steel tube with a defined mass (deuterated derivative of toluene - 40 ng per tube) of standard solution was analyzed employing the same analytical protocol as for the polymeric samples. Considering the obtained results of analytes recovery studies, recovery values range from 96 to 104%. Making

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an allowance for the equation of the calibration curve, the numerical values of method quantification limit (MQL) of the employed analytical protocol was estimated at 0.45 ng/g with toluene d_8 as a reference compound. More detailed information about the QA/QC protocol might be found elsewhere (Marć et al., 2017; Marć and Zabiegała, 2017).

The mathematical approach is based on best-fit function modeling, with ecotoxicity or TVOC concentration as dependent variable *Y* and time as independent variable *X*. As in previous studies on ecotoxicity modeling, the most appropriate model seems to be the polynomial fit of the 'Y = $a_1X^2 + a_2X + b$' type, which accurately describes the experimental data. The weight coefficient $\boldsymbol{a_1}$ (by value and by sign) can be used to interpret the impact of time. Model validation was performed using R^2 values (multiple correlation coefficients).

3. Results and Discussion

3.1. Toxicological studies

3.1.1. Impact of time on toxicity of aqueous extracts

In Table 1a, the values of a_1, a_2, b and R^2 are presented for all 10 models (refer to Figure 1, 10 different objects of study – parts of the original brand product, numbers in front of object names denote extraction time). In almost all cases (9 out of 10 objects) $\boldsymbol{a_1}$ is negative, which is an indication that, in general, toxicity in water decreases with time. The complex nature of the toxicity/time relationship, however, is indicated by the appearance of local maxima and minima in the time dependence model. Strong time dependence is observed for objects/samples 1 Foil B, 2 Foil B, 6 Foil B, 12 Foil B, 1 Foil NB, and 2 Foil NB, with low time dependence for 0.5 Foil B, 0.5 Foil NB, 6 Foil NB and 12 Foil NB (foil and chocolate; sample 0.5 Foil NB showed very low time impact). Model adequacy is acceptable and especially high for samples 1 Foil B, 2 Foil B, 6 Foil NB and 12 Foil NB. In Table 2, the ecotoxic effects in water for all cases and time periods are presented. The highest toxicity is observed among the "chocolate" cases, while the lowest is observed for case 1 Foil NB (the strong time impact found for this case is related to the significant changes of effect from the starting time to the final time).

3.1.2. Impact of time on toxicity of artificial saliva extracts

In Table 1b, the values of a_1, a_2, b and R^2 are presented for all 10 models (10 different objects of study) for the artificial saliva medium. For this medium, the models are less adequate. As

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seen in Table 2, the ecotoxicity values are much lower than those in water (with the exception of cases 6 Foil NB and 12 Foil NB – extracts of "chocolate" objects that correspond almost entirely to the models in the water medium). All other cases (0.5 Foil B, 1 Foil B, 2 Foil B, 6 Foil B, 12 Foil B, 0.5 Foil NB, 1 Foil NB, 2 Foil NB) differ both in absolute value of ecotoxic effects and time trends. Samples 12 Foil B and 2 Foil NB do not indicate a significant time trend. Models of low adequacy are found for samples 0.5 Foil B, 1 Foil B, and 6 Foil B. For the remaining cases, two (2 Foil B and 0.5 Foil NB) show a local minimum of toxicity at 2 hours of treatment while one (1 Foil NB) shows local maximum at 2 hours of treatment. Thus, no reliable interpretations of time impacts are reasonable. However, one interesting finding is the response of the "chocolate" extract samples. The toxicity data for artificial saliva extracts are surprising in some extent when increasing contact time leads to increased bioluminescence inhibition, it suggests that composition of simulation media used enhances extractability of xenobiotics present in chocolate material making the solution more toxic (impact of time is also noticeable). There are studies being performed aimed at identification of substances plausibly responsible for observed elevated toxicity levels.

3.2. TVOC results

For 6 samples (2 Capsule B, 6 Capsule B, 12 Capsule B, 0.5 Capsule NB, 1 Capsule NB, 2 Capsule NB), TVOC analyses were performed. The results are interpreted using the method described for Microtox effects. Polynomial models proved to be most satisfactory for the results obtained. In Table 1c, the calculated parameters of the polynomial model are presented. The values of TVOC emissions for all objects studied and all time periods are listed in the Table 3. An attempt was made to compare both the models and the experimental values for Microtox toxic effects (in water and artificial saliva) and TVOC concentrations for the same objects. As presented in Table 3, there is a significant difference between the emitted TVOCs for objects 2 Capsule B and 0.5 Capsule NB when compared to 1 Capsule NB and 2 Capsule NB. The polynomial model satisfactorily describes all samples except for 12 Capsule B, which is characterized by high TVOC concentrations for very short and very long times and minima for intermediate times. Specific to these models is the low value for a_1 and very high values for a_2. Thus, the a_2 value is considered a better descriptor of the time trend. Therefore, for 2 Capsule B and 6 Capsule B samples, TVOC concentration generally decreases with time, while 12 Capsule B, 1 Capsule NB and 2 Capsule NB do not show significant time trends since 0.5 Capsule NB reveals a concentration increase with time.

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Taking into account the information listed in the Table 3 it was noticed a clear difference in assessed TVOC parameters between original brand products and non-brand products. It was observed that in both cases (small toys and containers) the original brand products were characterized by much higher TVOCs emission values than studied non-brand products. To explain the occurrence of this type of phenomenon, it was necessary to conduct the FT-IR analysis of the brand and non-brand products. This solution gives a possibility to define the main polymer material from which studied products were made. The results of FT-IT analysis of brand and non-brand products were present on the Supplementary Figures from 3 to 5. Considering the obtained results of FT-IR analysis of small toys and containers, both original brand and non-brand products, and comparing them to the FT-IT software database, it was conclude that the TVOCs emissions might be indicated by two main factors: (i) the pigment application (sample color) and (ii) the type of the main polymer material which was used at the studied product manufacturing process. In a first case, the polymer containers defined as Capsule NB and Capsule B were mainly made of a mixture polypropylene + polyethylene-p (according to performed FT-IR analysis) – in this situation, the main polymer material was the same, but the color of containers was different. Due to this fact it might be pointed out that the application of defined pigment might influence on the TVOCs emission values. As for the second case (small toys), the higher TVOCs emissions observed for original brand products might be caused by the type of polymer that was used. The original brand products (Toy 1B and Toy 2B) were mainly made of acrylonitrile-butadiene-styrene (ABS) copolymer. As for the non-brand products (Toy 1NB and Toy 2NB), the small toys were mainly made of polyethylene (Mn 1800). The ABS copolymer is characterized by much higher TVOCs emissions due to the fact, that during a manufacturing process, tree types of polymers/reagents must be used to obtain the desired copolymer. Taking into consideration this information, the presence of the mentioned phenomena of TVOCs emissions, might be caused by the type of the polymer which was used at the manufacturing process of the brand and non-brand products.

3.3. XenoScreen YES/YAS results

Results of endocrine potential studies are presented in Table 4. In most cases, only androgenic antagonistic behavior of extracts was observed, proving the objects studied to be a major threat in relation to this endpoint. As other endpoints (oestrogenic agonistic, oestrogenic

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antagonistic, and androgenic agonistic) showed no significant values or variations, no models could be calculated.

3.4. Comparison between Microtox® ecotoxic effects and TVOC concentrations

The comparison between Microtox® ecotoxic effects and TVOC concentrations was performed by correlation analysis. In the first run, correlation between all water medium effects with all artificial saliva effects resulted in a correlation coefficient of 0.63. This result indicates that both series of effects are relatively well correlated, probably due to the very high correlation between samples 6 Capsule NB and 12 Capsule NB in both series. In Table 5, the correlation coefficients for the series MT water/TVOC and MT saliva/TVOC are presented. Assuming that correlation coefficients (as absolute value) below 0.3 are insignificant and between 0.31 – 0.60 are of relatively low significance, one might conclude that reasonable correlation (negative) is detected for cases 2 Capsule B and 12 Capsule B (MT water/TVOC) and for case 6 Capsule B (positive for MT saliva/TVOC).

4. Conclusions

The results from studies of both toxicological and instrumental analyses of TVOC demonstrate that there is a significant problem with assigning the health impact of products intended for children using purely instrumental methods. Although significant emissions of toxicants from toys were not observed, the toxicity of chocolate extracts was significant, demonstrating either its low quality or high capacity as a sink for volatile organic compounds emitted from materials in contact with it. The obtained results on acute toxicity of polymer material extracts coincide with data presented by other researchers. In studies where D. magna was used as biologically active element, no acute toxicity was detected in water extract of PE such as ABS (Lither et al., 2009, 2012). On this basis, it can be assumed that the compounds released from the surface of these materials are so in low concentration that they do not cause visible adverse effects in test organisms, or the exposure time was too short. It therefore seems necessary to check whether this toxicity occurs after prolonged exposure (chronic toxicity). As for the general law regulations about the organic compounds emission from plastic materials directly into the food products, in the EU there is document defined as non-legislative acts - the Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. In the mentioned regulation,

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the union list of authorised monomers, other starting substances, macromolecules obtained from microbial fermentation, additives and polymer production aids was attached. Moreover, mentioned document defines only a numerical values of SML [mg/kg] factor, which is the specific migration limit applicable for the substance, expressed in mg substance per kg food. There is a lack of general information about the limits of TVOCs emissions form plastic materials which have direct contact with food products.

More alarming conclusions can be withdrawn from results on endocrine potential studies, as the androgenic impact of extracts is clearly observable on samples extracted from plastic toys and chocolate. These results may indicate that compounds such as phthalates, bisphenol A or compounds from the group of brominated flame retardants are released from the surface of the materials. In *in vitro* conditions, all of these compounds have been found to exhibit anti-androgenic activity (Wooten et al., 2013, Kharlyngdoh et al., 2015). Moreover, many studies can be found in literature confirming that it is these groups of analytes that are mainly leached from the surface of children's toys (Nerev et al., 2018, Ionas et al., 2014). The presence of weak anti-estrogenic responses in some samples may indicate that other xenobiotics are also released from the surface of the materials. Lack of significant correlations between instrumental and biological assay data may also be associated with the occurrence of synergy or antagonism effect. To date, few studies have been carried out to assess the toxicity and endocrine nature of compounds migrating from children's toys. Further research is needed to prove the applicability of bioassay studies in assessing the impact of xenobiotics present in materials intended for kids or as a validated tool in product quality assessment. Clear discrepancies between different commercially available products show that such an assessment is plausible, while more environmentally benign than many instrumental methods.

In TVOC emission profile studies, a similar characteristic of the emission profile typical of the new type of plastic indoor materials was observed - a decrease in the TVOC emission rate along with an increase in the storage/seasoning time inside the environmental chamber. This type of relationship was observed for all studied samples of children’s toys and plastic containers (Marć et. al., 2015, 2017, Tirendi et. al., 2009). This may indicate that the chemicals released from polymeric materials can be present in the gaseous phase between the small polymeric material and the chocolate food product and might be adsorbed or dissolved in the chocolate, directly affecting the human body. Additionally, significant amounts of harmful organic compounds might be released directly to the gaseous phase from the surface of plastic packaging, which has direct contact with the chocolate food product. However, it should be clarified that further, more detailed studies are needed to define precisely the

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individual chemicals that are emitted from the surface of plastic samples directly into the gaseous phase – indoor environment. Defining the TVOC parameter or TVOC emissions profile allows researchers to obtain screening information that provides the opportunity to highlight potential problems. The future research, associated with the described interdisciplinary studies, ought to be focused on the assessing the emission rates of selected carcinogenic compounds or endocrine-disrupting chemicals that might be released from plastic materials and have direct contact and interaction with chocolate food products.

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6. Conflict of interest

The authors declare that they have no conflict of interest.

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Figure Captions

Figure 1. Figure 1. Schematic representation of samples studied (legend for results description: foil B, foil NB – aluminum foil package for brand and non-brand product, respectively; capsule B, capsule NB – plastic capsule/package inside chocolate to cover toy, for brand and non-brand product, respectively; toy 1B, toy 2B,– toys inside plastic package of brand original product; toy 1NB, toy 2NB – toys inside plastic package of non-brand (fake) product; chocolate B, chocolate NB – brand (original) and non-brand (fake), respectively, chocolate sample being in contact with aluminum foil)

Supplementary Figure and Tables:

Supplementary Figure 1. Analytical procedure of Microtox® acute toxicity determination.

Supplementary Figure 2. Diagram of the procedure used in order to assess the oestrogenic and androgenic activity.

Supplementary Figure 3. The general view of FT-IR spectra of plastic container/capsules.

Supplementary Figure 4. The general view of FT-IR spectra of small polymeric toys - original brand products.

Supplementary Figure 5. The general view of FT-IR spectra of small polymeric toys – non-brand products.

Supplementary Table 1. General information about the working conditions of applied analytical equipment and techniques during the conducted TVOCs research

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Table 1. Values of the regression coefficients and correlations.

Extraction time [h] / object name	a_1	a_2	R^2
a)			
0.5/ Foil B	-0.14	1.55	0.1
1/ Foil B	-4.71	31.38	0.91
2/ Foil B	-4.86	33.84	0.78
6/ Foil B	-3.11	27.21	0.44
12/ Foil B	-4.72	30.18	0.52
0.5/ Foil NB	0.58	6.17	0.57
1/ Foil NB	-3.79	25.41	0.40
2/ Foil NB	-3.30	20.30	0.29
6/ Foil NB	-1.43	23.27	0.91
12/ Foil NB	-0.20	15.49	0.85
b)			
0.5/ Foil B	-4.33	29.16	0.28
1/ Foil B	1.65	-6.19	0.17
2/ Foil B	4.21	-24.63	0.48
6/ Foil B	-1.53	5.02	0.21
12/ Foil B	-0.07	-1.89	0.1 ***ns***
0.5/ Foil NB	3.27	-22.34	0.68
1/ Foil NB	-0.87	1.27	0.61
2/ Foil NB	0.63	-4.49	0.1 ***ns***
6/ Foil NB	-1.94	21.07	1.00
12/ Foil NB	-1.63	19.32	0.83
c)			
2/ Capsule B	499.9	-3400	0.58
6/ Capsule B	103.3	-1373	0.84
12/ Capsule B	-463.8	8415	0.1 ***ns***
0,5/ Capsule NB	-679.1	5700	0.66
1/ Capsule NB	44.9	-214	0.95
2/ Capsule NB	63.9	291	0.65

Table 2. Results of Microtox® toxicity effect in water and artificial saliva extracts

Extraction time [h] / object name	Water extraction		Artificial saliva extraction	
	Bioluminescence inhibition [%]	SD	Bioluminescence inhibition [%]	SD
0.5/ Foil B	5.862	0.023	-25.427	0.035
1/ Foil B	23.620	0.043	7.881	0.039
2/ Foil B	23.596	0.025	-20.376	0.039
6/ Foil B	15.508	0.039	15.669	0.015
12/ Foil B	14.445	0.019	-13.512	0.030
0.5/ Foil NB	13.409	0.001	-13.219	0.033
1/ Foil NB	37.354	0.010	17.876	0.018
2/ Foil NB	40.539	0.015	-11.181	0.040
6/ Foil NB	35.199	0.016	-8.220	0.013
12/ Foil NB	30.547	0.003	18.427	0.039
0.5/ Capsule B	-0.531	0.036	-22.855	0.027
1/ Capsule B	13.689	0.004	-28.576	0.014
2/ Capsule B	20.499	0.019	-49.231	0.085
6/ Capsule B	35.744	0.006	-22.879	0.112
12/ Capsule B	11.693	0.022	-22.661	0.028
0.5/ Capsule NB	2.412	0.019	-20.924	0.015
1/ Capsule NB	36.889	0.036	9.142	0.029
2/ Capsule NB	13.770	0.025	-19.689	0.029
6/ Capsule NB	40.105	0.018	-32.164	0.042
12/ Capsule NB	22.618	0.028	-20.954	0.047
0.5/ Toy 1B	-0.531	0.036	-22.855	0.027
1/ Toy 1B	2.932	0.006	-22.932	0.050
2/ Toy 1B	32.879	0.007	3.896	0.020
6/ Toy 1B	15.819	0.030	-24.171	0.042
12/ Toy 1B	32.296	0.003	-32.720	0.038
0.5/ Toy 2B	12.407	0.001	-16.125	0.136
1/ Toy 2B	7.419	0.002	-7.737	0.032
2/ Toy 2B	23.828	0.034	-14.305	0.032
6/ Toy 2B	6.921	0.021	-20.661	0.065
12/ Toy 2B	52.023	0.033	-31.369	0.041
0.5/ Toy 1NB	6.298	0.031	-18.750	0.008
1/ Toy 1NB	29.836	0.048	-15.454	0.034
2/ Toy 1NB	13.347	0.026	-16.947	0.026
6/ Toy 1NB	39.166	0.016	-34.117	0.006
12/ Toy 1NB	15.036	0.018	-29.046	0.029
0.5/ Toy 2NB	-9.460	0.026	-21.009	0.024
1/ Toy 2NB	24.147	0.013	-12.907	0.028
2/ Toy 2NB	3.728	0.031	-22.862	0.018
6/ Toy 2NB	-[1]	-	-26.985	0.100
12/ Toy 2NB	3.669	0.058	-17.358	0.036
0.5/ Chocolate B	15.912	0.056	26.540	0.056
1/ Chocolate B	19.891	0.157	42.277	0.054
2/ Chocolate B	47.140	0.012	52.922	0.011
6/ Chocolate B	68.144	0.010	61.003	0.019
12/ Chocolate B	65.233	0.022	64.473	0.019
0.5/ Chocolate NB	25.556	0.031	41.463	0.019
1/ Chocolate NB	20.311	0.096	51.217	0.011
2/ Chocolate NB	51.280	0.017	58.282	0.009
6/ Chocolate NB	72.615	0.005	83.284	0.038
12/ Chocolate NB	70.762	0.008	72.516	0.006

[1] Unrepeatable results, omitted in data treatment

Table 3

Table 3. TVOCs emission values for all cases and times studied.

object	Sample mass [g]	Sample seasoning time inside a chamber [h]	TVOC emission [ng·g^{-1}]
Capsule B	3.9902	0.5	3273.24
		1	2610.25
		2	2886.47
		6	2280.37
		10	2935.57
Capsule NB	3.8232	0.5	1114.40
		1	1123.72
		2	620.06
		6	564.16
		10	538.73
Toy 1B	6.8347	0.5	1420.44
		1	384.33
		2	440.33
		6	343.14
		10	1109.06
Toy 2B	7.3696	0.5	210.72
		1	1252.19
		2	1211.94
		6	986.86
		10	1484.63
Toy 1NB	1.8211	0.5	96.91
		1	88.05
		2	79.62
		6	100.55
		10	183.46
Toy 2NB	2.9535	0.5	415.57
		1	310.08
		2	280.00
		6	560.19
		10	545.37

Table 4

Table 4. Results of endocrine potential determinations for extracts.

Extraction time [h] / object name	YES +		YES -		YAS +		YAS -	
	water	artificial saliva	water	artificial saliva	water	artificial saliva	water	artificial saliva
0.5/ Foil B	0	0	0	1	0	0	2	2
1/ Foil B	0	0	0	0	0	0	3	0
2/ Foil B	0	0	0	0	0	0	2	5
6/ Foil B	0	0	0	2	0	0	5	4
12/ Foil B	0	0	0	0	0	0	6	3
0.5/ Foil NB	0	0	0	1	0	0	2	2
1/ Foil NB	0	0	0	0	0	0	3	1
2/ Foil NB	0	0	0	2	0	0	2	5
6/ Foil NB	0	0	0	2	0	0	5	5
12/ Foil NB	0	0	0	1	0	0	3	3
0.5/ Capsule B	0	0	1	0	0	0	2	1
1/ Capsule B	0	0	0	0	0	0	0	1
2/ Capsule B	0	0	0	0	0	0	2	3
6/ Capsule B	0	0	0	2	0	0	4	5
12/ Capsule B	0	0	1	0	2	0	6	3
0.5/ Capsule NB	0	0	0	1	2	0	1	2
1/ Capsule NB	0	0	0	0	0	0	0	1
2/ Capsule NB	0	0	0	0	0	0	2	0
6/ Capsule NB	0	0	0	0	0	0	4	3
12/ Capsule NB	0	0	0	0	2	0	6	2
0.5/ Toy 1B	0	0	1	1	0	0	2	0
1/ Toy 1B	0	0	0	0	0	0	3	2
2/ Toy 1B	0	0	0	0	0	0	2	2
6/ Toy 1B	0	0	0	0	0	0	4	5
12/ Toy 1B	0	0	0	0	0	0	3	0
0.5/ Toy 2B	0	0	1	1	0	0	2	1
1/ Toy 2B	0	0	0	0	0	0	3	2
2/ Toy 2B	0	0	0	0	0	0	2	4
6/ Toy 2B	0	0	0	0	0	0	4	5
12/ Toy 2B	0	0	0	0	0	0	2	3
0.5/ Toy 1NB	0	0	2	0	0	0	2	3
1/ Toy 1NB	0	0	0	0	0	0	3	1
2/ Toy 1NB	0	0	0	0	0	0	2	5
6/ Toy 1NB	0	0	0	0	0	0	4	0
12/ Toy 1NB	0	0	1	0	2	0	6	3
0.5/ Toy 2NB	0	0	1	0	0	0	2	2
1/ Toy 2NB	0	0	0	0	0	0	2	2
2/ Toy 2NB	0	0	0	0	0	0	2	5
6/ Toy 2NB	0	0	0	0	0	0	2	2
12/ Toy 2NB	0	0	0	0	0	0	2	2
0.5/ Chocolate B	0	0	0	0	0	0	2	3
1/ Chocolate B	0	0	0	0	0	0	2	2
2/ Chocolate B	0	0	0	0	0	0	4	5
6/ Chocolate B	0	0	0	0	0	0	5	3
12/ Chocolate B	0	0	0	0	0	0	4	3
0.5/ Chocolate NB	0	0	0	0	0	0	2	3
1/ Chocolate NB	0	0	0	0	0	0	2	2
2/ Chocolate NB	0	0	0	2	0	0	3	5
6/ Chocolate NB	0	0	0	0	0	0	5	4
12/ Chocolate NB	0	0	0	0	0	0	4	3

Explanation of numbers

0	1	2	3	4	5	6
Weak effect			→			**Strong effect**

Table 5

Table 5. Correlation coefficients between bioluminescence effects for different times in relation to TVOC emissions for the same times

Objects	Water media	Artificial saliva
Capsule B	**-0.90**	-0.07
Capsule NB	-0.22	**0.69**
Toy 1B	**-0.88**	-0.10
Toy 2B	0.43	-0.29
Toy 1NB	-0.17	-0.54
Toy 2 2NB	-0.37	-0.37

Figure 1

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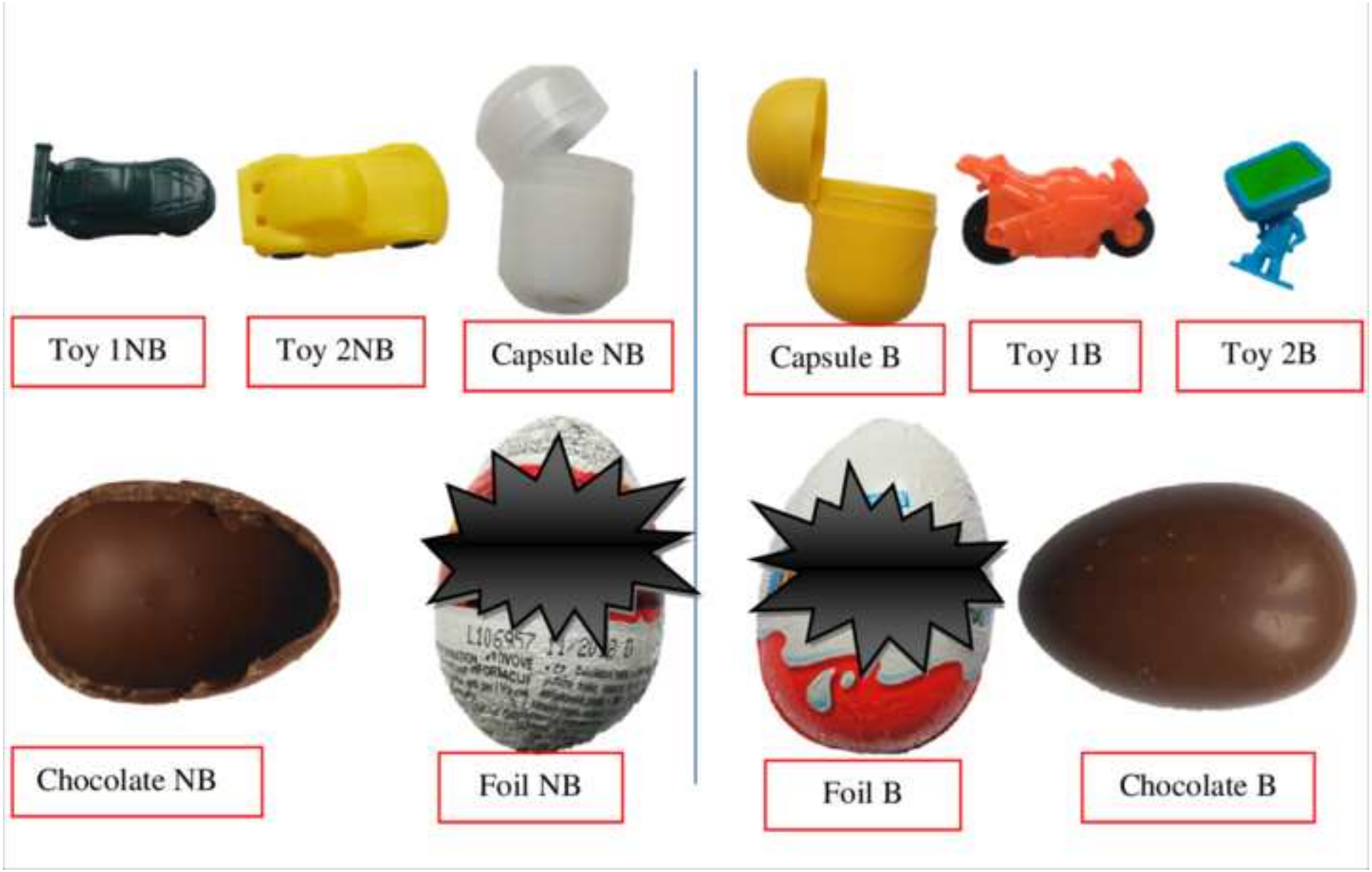

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S.1. Materials and methods

S.1.1. Chemicals and reagents

Chemicals used in the research were: sodium chloride (Sigma Aldrich, Germany), dipotassium phosphate (Ciech S.A., Poland), calcium chloride (Eurochem BGD, Poland), magnesium chloride, potassium chloride, potassium carbonate, lactic acid, urea (POCH S.A., Poland), acetic acid (35-38% w/w), ammonium hydroxide (25 % w/w), (Chempur, Poland), distilled water. Microtox® 500 kit (Microtox Diluent, 2 % NaCl, lyophilized *Vibrio fischeri*, Osmotic Adjusting Solution (OAS), Microtox Acute Reagent, Reconstitution Solution (RS) was purchased from ModernWater Ltd. (GB). Ethanol (EtOH, CAS no. 64-17-5), acetic acid (CAS no. 64-19-7), dimethyl sulfoxide (DMSO, CAS no. 67-68-5), and Parafilm® were purchased from Sigma-Aldrich (Germany). All reagents were of analytical grade or higher. XenoScreen YES/YAS reagents (CPRG (chlorophenol red-β-D-galactopyranoside), basal medium, copper sulfate, L-aspartic acid, L-threonine, hAR and hER yeast cells stabilized on the filtration paper, vitamin, vials with calibrators: 5α-dihydrotestosterone, 17β-estradiol, flutamide, 4-hydroxytamoxifen) were purchased from Xenometrics G. A. (Switzerland). culture flasks with gas-permeable filter caps, gas-permeable plate sealers, 96-well plates, were purchased from GenoPlast Biochemicals (Poland). The equipment and instruments used were: Microtox® 500 (Modern Water Ltd. (GB)), electronic multi- and single-channel pipettes (Eppendorf (Germany)), CP411 pH-meter (Metron (Poland)), shaker type water bath 357 (Elpan Laboratory Instruments (Poland)).

Supplementary Table 1

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Supplementary Table 1. General information about the working conditions of applied analytical equipment and techniques during the conducted TVOCs research

Sampling stage of analytes emitted from the surface of studied polymer products to the gaseous phase.	
Device applied to seasoning/conditioning samples of polymer products	Markes' Micro-Chamber/Thermal Extractor™ – µ-CTE™ 250 (*Markes International Inc., Gold River, California, USA*) containing four microscale chambers
Internal volume of a single chamber	114 cm^3
Seasoning/conditioning temperature	40° C
Conditions for conditioning/seasoning samples inside the chambers - procedure applied for collecting the organic compounds samples from gaseous phase	Seasoning/conditioning of polymer products (children's toys samples and plastic containers) under static conditions, without forced flow rate of inert gas through the chambers; Seasoning/conditioning at predefined time intervals: 0.5 h, 1.0 h, 2 h, 6 h, 10 h – starting from the moment when the studied samples were placed inside the chambers.
Nitrogen flow rate during the stage of collecting the organic compounds samples	50 ml/min
The elution time of the gaseous phase from the chamber to the sorption medium after a defined conditioning/seasoning time	25 min
Tool used to collect the organic compounds samples from gaseous phase	Cylindrical container filled with defined amount of sorption medium Tenax TA, 35/60 mesh (*Markes International Ltd*.) containing specify amount of internal standard solution (deuterated derivative of toluene - 40 ng per tube)
The liberation stage of analytes retained on a sorption medium (Tenax TA)	
Device employed to liberate the organic compounds from the sorption medium	Two-stage thermal desorber (*Omnisfera S.C., Gdansk, Poland)*
The 1st stage of thermal desorption heating temperature	280° C Microtrap* temperature – 0°C
The heating period of a Tenax TA tube	15 min
Helium flow rate passing through the Tenax TA tube	40 ml/min
The 2nd stage of thermal desorption heating temperature	Microtrap temperature - 300° C
Microtrap ballistic heating period	5 min
Helium flow rate passing through the microtrap in a straight line to the GC column.	2.2 mL/min
The conditions of final determination stage of analytes thermally extracted from the sorption medium	
Gas chromatography system	Hewlett-Packard 5890 GC Series II
Detector	Flame Ionisation Detector
Detector temperature	250° C
TD-GC transfer line temperature	150° C
Capillary column type	DB-1 (*J&W*) 30 m x 0.32 mm x 5 µm
Carrier gas type (flow rate)	Helium (2.2 mL/min)
Oven temperature program	40° C by 1 min; 5° C/min up to 125° C; 10° C/min up to 220° C hold 220° C by 5 min
** the microtrap tube is filled with two types of sorption medium - Carbotrap (approx. 27 mg) and Tenax TA (approx. 37 mg).*	

Supplementary Figure 1

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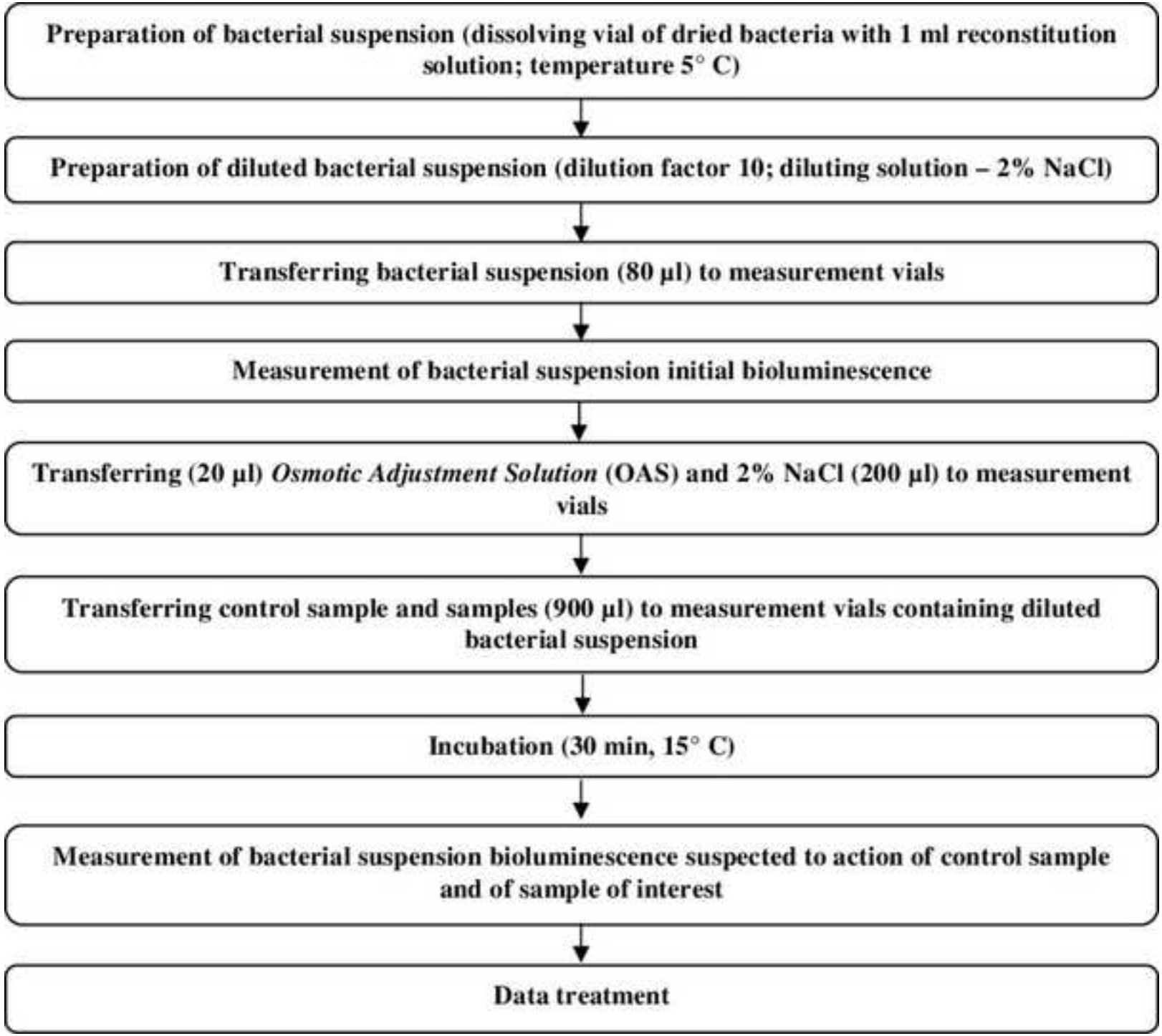

Supplementary Figure 2

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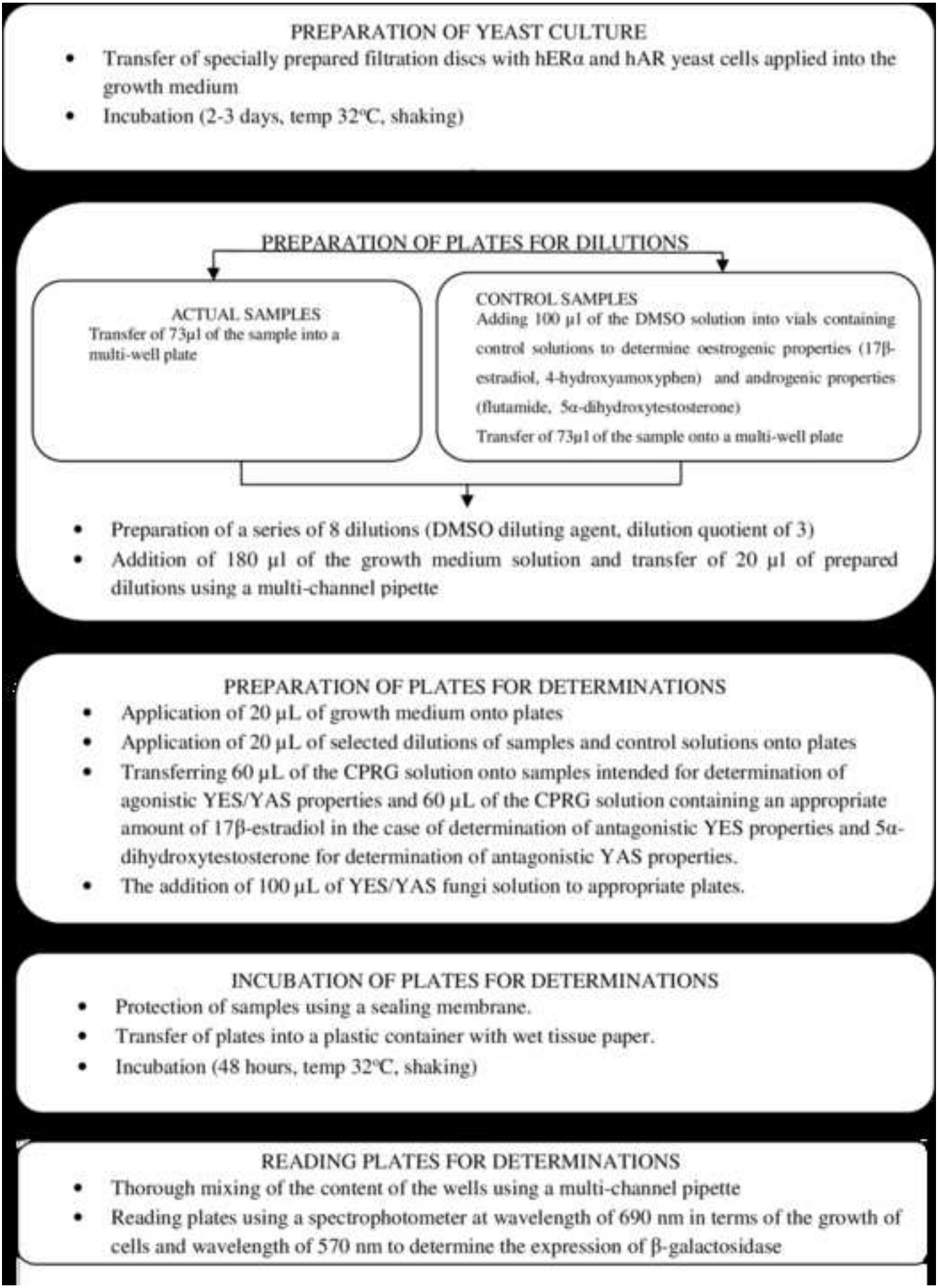

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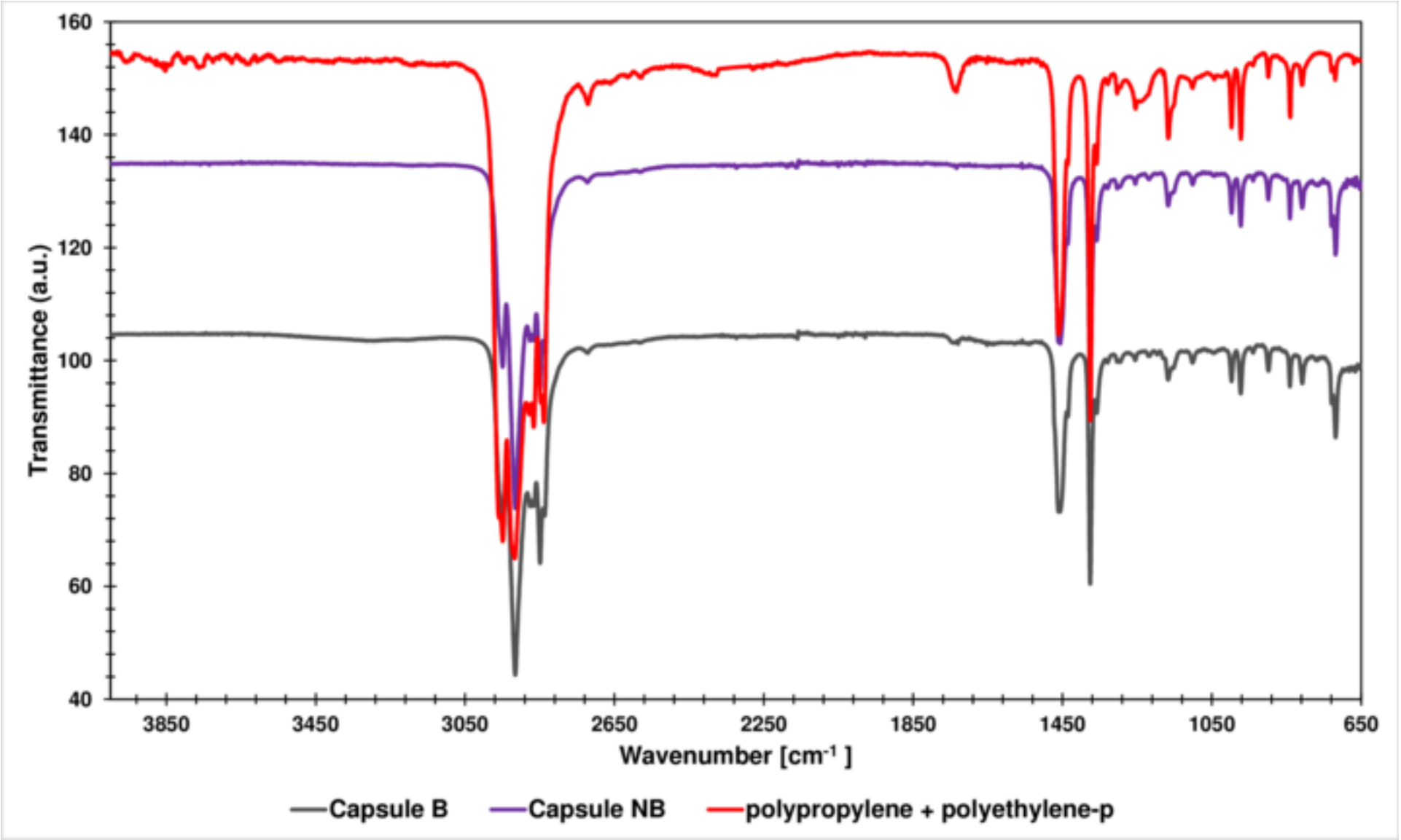

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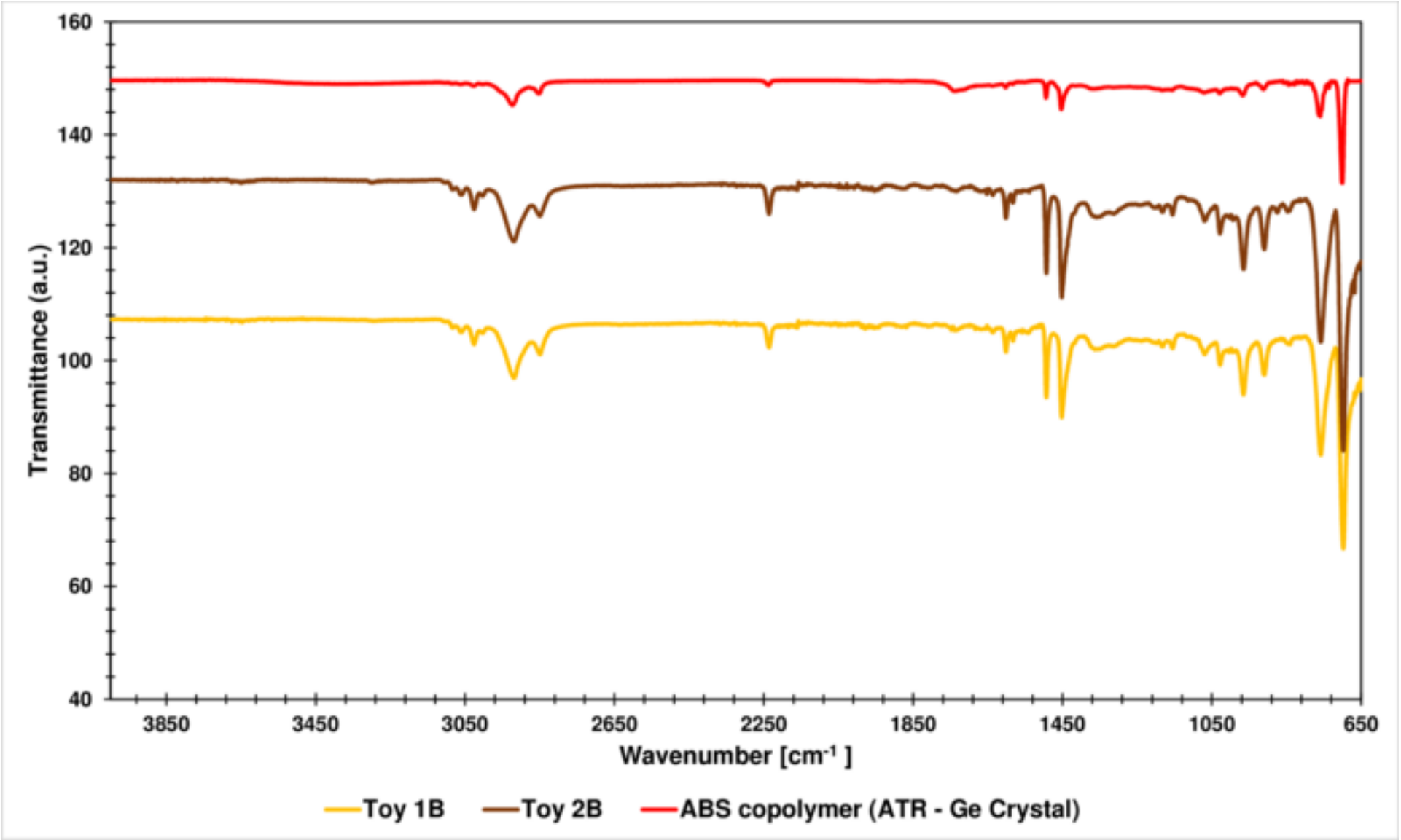

Supplementary Figure 5

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