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Levels of methyleugenol and eugenol in instant herbal beverages available on the Indonesian market and related risk assessment

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ABSTRACT

The presence and accompanying risks of methyleugenol and eugenol in herbal beverages available on the Indonesian market were evaluated. Methyleugenol was detected in 49 out of 114 samples, at levels amounting to 2.6–443.7 μg/g, while 4 samples contained eugenol at 21.4–101.2 μg/g. The EDI resulting from drinking these preparations amounted to 0.1–51.2 μg/kg bw/day and 1.1–3.3 μg/kg bw/day, respectively for samples targeted at adults and children. A $BMDL_{10}$ value of 22.2 mg/kg bw/day for methyleugenol was defined using literature data and model averaging. MOE values were below 10,000 for 46 samples (40.4%), indicating a priority for risk management when assuming daily lifelong consumption, while the EDI for 4 samples containing eugenol did not exceed the ADI of 2.5 mg/kg bw thus did not raise a concern for human health. Using Haber's rule to correct for less than lifetime exposure, consumption of methyleugenol via these beverages would be of low concern when consumed for less than 2 weeks/year during a lifetime. This conclusion holds for herbal beverages collected by targeted sampling, not for all herbal beverages on the Indonesian market. The study provides data that can support establishment of a maximum permitted level (MPL) for methyleugenol in herbal beverages in Indonesia.

1. Introduction

Herbal beverages can be enriched in herbs such as ginger (*Zingiber officinale* Rosc), cloves (*Syzygium aromaticum* L.), cinnamon (*Cinnamomum burmannii* Blume), fennel (*Foeniculum vulgare* Mill.), betel pepper (*Piper betle* L.), star anise (*Illicium verum*), nutmeg (*Myristica fragnan*) or other herbs. Generally, such enrichment of herbal beverages with selected or mixed herbs is aiming at possible beneficial health effects (Muhammad and Dewettinck, 2017). Ginger containing beverages including preparations like *wedang jahe*, *bandrek*, *ronde*, *bajigur*, and *sekoteng*, are among the most popular herbal beverages consumed in Indonesia, because ginger has a long history as a thermogenic and antiemetic drink (Bryer, 2005). Traditionally, Indonesian people need to prepare the herbal beverages by using mixed fresh herbs, but nowadays more people tend to choose instant herbal beverages which are commercially available. These products are usually in powder form, packaged in sealed and labelled plastic sachet, and readily consumed upon adding hot water and then drinking the resulting preparation.

Nowadays, consuming instant herbal beverages in Indonesia shows an increasing trend due to the easy online access and the fact that websites provide information about all kind of possible beneficial health effects of these products including: maintaining and increasing health, prevention of diseases, counteracting cold, increasing vitality after work, and slimming and cosmetic effects especially for women (NADFC, 2017). In addition, the majority of people consider these herbal beverages to be safe, even during pregnancy, with less side effects than conventional medicines (Hassali et al., 2009). The limitation of knowledge related to possible adverse health effects of herbal beverages may put consumers at risk that may arise from (over)consumption of herbal beverages, and therefore the quality and safety of these products should be assessed and monitored (Butt and Sultan, 2013).

The safety regulation of herbal products varies internationally among jurisdictions (Low et al., 2017). Herbal products are classified by the US Food and Drug Administration (FDA) as dietary supplements or foods and are marketed pursuant to the Dietary Supplement Health and Education Act (DSHEA) of 1994. This implies that a dietary supplement cannot carry any health claim or medical advice on the label (FDA, 2017). Similarly, in the European Union botanical preparations are

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List of abbreviations

ACCM	Advisory Committee on Complementary Medicines
ADI	Acceptable Daily Intake
AFB1	Aflatoxin B1
AIC	Akaike information criterion
BMD	Benchmark dose
$BMDL_{10}$	Lower confidence limit of the benchmark dose resulting in a 10% extra cancer incidence
BMDU	Upper confidence bound of the BMD
DSHEA	Dietary Supplement Health and Education Act
EDI	Estimated daily intake
EU	European Union
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
INARAC	Indonesia Risk Assessment Center
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MOE	Margin of Exposure
MoH RI	Ministry of Health of Republic Indonesia
MRA	Microbiology Risk Assessment
NADFC RI	National Agency for Drug and Food Control Republic of Indonesia
RIFM	Research Institute for Fragrance Materials
UPLC	Ultra Performance Liquid Chromatography

considered food and these preparations should comply with broader requirements as defined for safe food while any health claim used needs scientific evaluation and approval by the European Food Safety Authority (EFSA) (Commission, 2008). Australia formed the Advisory Committee on Complementary Medicines (ACCM) in 2010 to address regulatory issues regarding the safety, efficacy and manufacturing quality of herbal remedies (TGA, 2017). In Indonesia, botanical preparations can be registered as food (NADFC, 2016b) but also as traditional medicine (MoH, 2012).

According to the Government Regulation 28/2004 on Food Safety, Quality and Nutrition, in Indonesia the food producer will receive a register approval number and get the marketing authorization number after passing the standard of safety and quality (Government-RI, 2004). The product registration type is based on two parameters including: (1) the safety regulatory body in Indonesia where the product is registered, being either BPOM RI (*Badan Pengawas Obat dan Makanan Republik Indonesia*/National Agency for Drug and Food Control Republic of Indonesia, NADFC RI) or Depkes RI (*Departemen Kesehatan Republik Indonesia*/Ministry of Health of Republic Indonesia, MoH RI) and (2) the category of the product being: MD (*Pangan Olahan Dalam Negeri*/Domestic Processed Food), ML (*Pangan Olahan Luar Negeri*/Foreign Processed Food), SD (*Suplemen Dalam Negeri*/Domestic Supplement), SL (Suplemen Luar Negeri/*Foreign Supplement*), TR (*Obat Tradisional Produksi Dalam Negeri*/Indonesian Traditional Medicine), TI (*Obat Tradisional Impor*/Imported Traditional Medicine), TL (*Obat Tradisional Lisensi*/Licensed Traditional Medicine), and P-IRT (*Pangan Industri Rumah Tangga*/Food Household Industry). So far, all products with the label MD, ML, SD, SL, TR, TI and TL were registered by BPOM RI, therefore they are labelled BPOM RI MD, BPOM RI ML, BPOM RI SD, BPOM RI SL and BPOM RI TR, BPOM TI, BPOM TL respectively, while the household food product were labelled by Depkes RI P-IRT (NADFC, 2016a).

The awareness and knowledge of herbal beverage producers in Indonesia related to the food safety and registration procedure is still limited (Putri, 2018; Yulianti, 2017). Therefore the risks of consumption of the herbal beverages produced may not be adequately evaluated and/or regulated or guaranteed. The Indonesia Risk Assessment Center (INARAC), a body under the NADFC RI, after its initiation in November 2014 so far presented a Microbiology Risk Assessment (MRA) of chicken Salmonella and a risk assessment on Aflatoxin B1 (AFB1) levels in peanuts and their processed products in 2016 (NADFC, 2017), while the safety and risks of frequent and prolonged consumption of herbal beverages have not yet been assessed.

Methyleugenol is a genotoxic and carcinogenic herbal ingredient that can be detected in basil (*Ocimum basilicum* L.) leaf (Bertoli et al., 2013), star anise (*Illicium anisatum* L.) fruit, bay Laurel (*Laurus nobilis* L.) leaf, and ginger (*Zingiber officinale* Rosc) rhizome (EFSA, 2009a). Recently, Suparmi et al. (2018) reported that methyleugenol was the major alkenylbenzene detected in most (91.3%) of the samples testing positive for the presence of alkenylbenzenes in Indonesian jamu. The consumer risk based on the Margin of Exposure (MOE) approach showed that the consumption of jamu for two weeks once every year during a whole lifetime of Indonesian people presents a priority for risk management for 5 out of 23 (21.7%) of the positively tested samples. Herrmann et al. (2013) reported that the exposure to methyleugenol leads to substantial levels of hepatic DNA adducts in the liver of human subjects. Twenty-nine out of 30 human liver samples were reported to contain the N^2-(*trans*-methylisoeugenol-3′-yl)-2′-deoxyguanosine adduct. And based on experimental animal studies the induction of liver carcinogenesis upon exposure to high dose levels of methyleugenol is well established (NTP, 2000).

Eugenol is another alkenylbenzene present in the herbs mentioned above and in the essential oils of botanicals frequently used in the herbal beverages including clove (*Syzygium aromaticum* L.), cinnamon (*Cinnamomum burmannii* Blume), and fennel (*Foeniculum vulgare* Mill.) (Api et al., 2016; Bakkali et al., 2008). In studies with eugenol in rats no carcinogenicity was observed while in a 2-year bioassay in mice the incidences of hepatocellular neoplasms were considered not significant and not dose-related (Maronpot et al., 1987; Miller et al., 1983; NTP, 1983; Rietjens et al., 2014). Also eugenol was considered not genotoxic at concentrations that did not result in cytotoxicity (EFSA, 2012b; JECFA, 2006; JECFA, 1982). This lack of genotoxicity and carcinogencity of eugenol resulted in establishment of an acceptable daily intake (ADI) of 0–2.5 mg/kg bw/day by JECFA (1982) and of 1.0 mg/kg bw/day by EFSA (2012b), while also a risk assessment by the Research Institute for Fragrance Materials (RIFM) Expert Panel concluded that eugenol does not present a concern for genetic toxicity in human health (Api et al., 2016). In line with this eugenol is listed as a permitted flavouring agent in the USA, EU, Australia and Indonesia (EFSA, 2009b; FDA, 2018; NADFC, 2018; TGA, 2007).

Based on these results it was anticipated that methyleugenol and eugenol may also be present and pose a risk in Indonesian instant herbal beverages. Therefore the objective of the present study was to analyse methyleugenol and eugenol in a large number (114 samples) of instant herbal beverages containing various mixed herbs collected on the Indonesian market by a targeted sampling strategy, and to perform an associated human risk assessment using the MOE approach for methyleugenol and the ADI for eugenol. Also an overview was made of their current product registration type indicated on the label. The results of this study may give information relevant for risk management aiming at prioritizing regulatory actions to reduce potential risks connected to instant herbal beverage consumption in Indonesia.

2. Materials and methods

2.1. Herbal beverage samples

A targeted sampling approach was applied, collecting herbal samples with the name of possible methyleugenol containing herbs on the label. These herbs included ginger (*Zingiber officinale* Rosc), cloves (*Syzygium aromaticum* L.), cinnamon (*Cinnamomum burmannii* Blume), fennel (*Foeniculum vulgare* Mill.), betel pepper (*Piper betle* L.), star anise (*Illicium verum*), nutmeg (*Myristica fragnan*), lemongrass (*Cymbopogon*

nardus L. Rendle), carrot (*Daucus carota* subsp. sativus), and galangal (*Kaempferia galanga*), all known to contain methyleugenol (Rietjens et al., 2014). One-hundred-fourteen samples of herbal instant beverages from different brands were purchased from traditional markets or supermarkets as depicted in Fig. 1, including sampling in Medan (1 store, $n = 2$), Semarang (11 stores, $n = 54$), Bawen (1 store, $n = 9$), Magelang (1 store, $n = 6$), Purworejo (1 store, $n = 1$), Yogyakarta (2 stores, $n = 9$), Surakarta (2 stores, $n = 7$), Nganjuk (4 stores, $n = 19$), Kediri (1 store, $n = 4$), and Mojokerto (1 store, $n = 3$).

Detailed information, including information on the respective herbs of concern present in the samples, the instructions for preparation and consumption of the beverages, beneficial effects claimed, and product registration according to the Indonesian system for labelling of these herbal samples outlined in the introduction, all as indicated on the label of each sample, are summarised in in supplementary Table 1. All samples, denoted B1-B114 in the present study, were in powder form and packaged in sachets. Among the 114 samples 98 samples were targeted to be consumed by adults, and 16 samples were dedicated to use by children.

2.2. Chemicals and reagents

Methyleugenol (purity 99%), estragole (purity 98%), safrole (purity 97%) and eugenol (purity 99%) were obtained from Sigma-Aldrich (Zwijdrecht, The Netherlands). Acetonitrile ULC/MS gradient, trifluoracetic acid (TFA) and dimethyl sulfoxide (DMSO) were purchased from Merck (Felsberg, Germany). Methanol ULC/MS gradient was purchased from Sigma-Aldrich, and nanopure water was obtained from an Arium pro UF/VF water purification system (Sartorius Weighing Technology GmbH, Gottingen, Germany).

2.3. Analysis of methyleugenol and eugenol levels

2.3.1. Methanol extract

The level of alkenylbenzenes present in the samples was determined by methanol extraction followed by UPLC analysis performed as described by Gursale et al. (2010) with minor modifications. The homogeneity of each sample was ensured by mixing manually 100 g from 6 to 20 sachets of sample in a ziplock plastic packet before taking samples for extraction. For the extraction methanol (10 ml) was poured over 1 g of homogenized sample in a brown glass extraction bottle, and the sample was mixed and sonicated for 15 min at room temperature. Upon sonication, the sample solution was filtered using a 0.45 µm syringe filter (VWR international). The filtrate was placed into a 1 ml Ultra Performance Chromatography (UPLC) vial and subsequently injected into the chromatographic system. Every sample was prepared and analysed in triplicate.

The extraction efficiency was evaluated based on the Guidance for Industry Bioanalytical Method Validation (2017) by spiking and mixing 1 g of sample B89, B114, with a methyleugenol standard dissolved in DMSO at a concentration that would give a final concentration of 10 µM upon addition of 10 ml methanol in the extraction procedure performed as described above. The average percentage of recovery was used to correct the levels of methyleugenol detected for the different instant herbal beverages samples. To confirm linearity of the method, samples were analysed in five different ratios of weight per volume of methanol in the range from 2.5 to 40% (w/v). In a separate recovery experiment the samples B89, B114 were also spiked with a mixture of eugenol and methyleugenol to determine recoveries. Eugenol was included in the studies because some samples appeared to contain eugenol (see result section). Since safrole and estragole were not detected in the respective samples their recovery was not further quantified. The limits of detection (LOD) amounted to 1.2, 3.0 and 5.5 µM for methyleugenol, safrole and estragole, respectively.

2.3.2. UPLC analysis

The methanol extracts were analysed in undiluted form. To this end 3.5 µl of each sample was subjected to UPLC analysis performed as described before (Van Den Berg et al., 2011). The UPLC used was a UPLC-DAD system consisting of a Waters (Milford, MA) Acquity binary solvent manager, sample manager, and photodiode array detector, equipped with a Waters Acquity UPLC BEH RP 18 column (1.7 µm, 2.1 × 50 mm). The column was kept at 22 °C, while the sample temperature was set at 10 °C. UPLC analysis was performed using a mobile phase A of nanopure water containing 0.1% (v/v) TFA and mobile phase B consisting of acetonitrile and a gradient program with a flow rate of 0.6 ml/min. The mobile phase composition started with 30.5% B, which was maintained for 15 min, followed by an increase to 80% B over 1 min and holding this for 0.5 min, followed by a decrease to 0% B in 1.5 min and keeping it at 0% for 1 min after which the eluents was returned to the initial conditions of 30.5% B for the next run in 20.5 min. Under the specified chromatographic conditions, the retention times for eugenol, methyleugenol, safrole and estragole were 2.2, 4.3, 8.1 and 9.0 min, respectively. Detection of safrole and estragole was done at 202 nm and used 225 nm respectively. For detection and quantitative analysis of methyleugenol, and eugenol, the wavelength was 202 nm. The peak intensity at this wavelength was compared to calibration curves of the compounds prepared using commercially available standards.

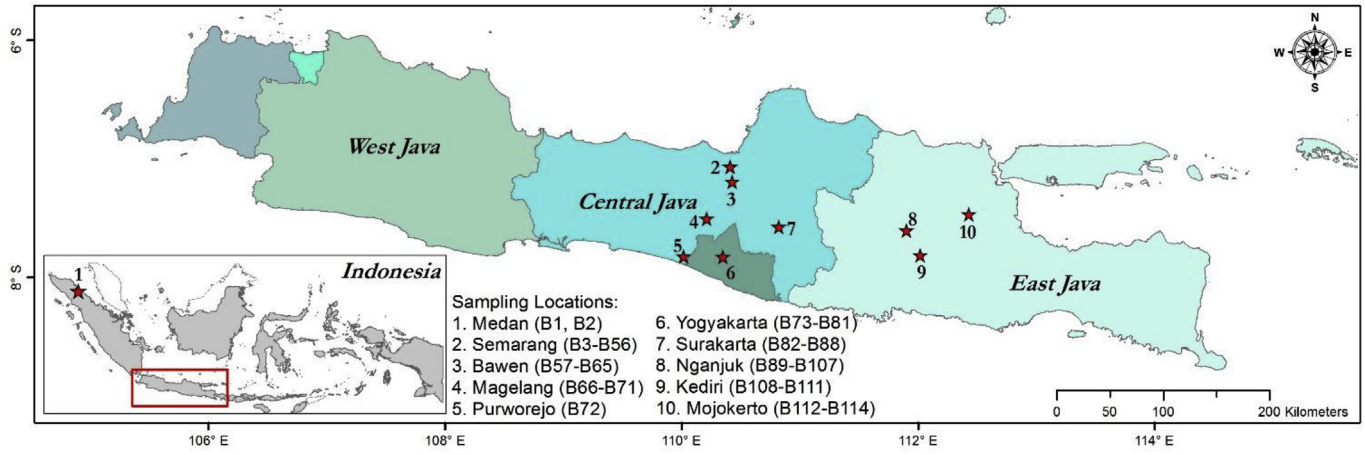

Fig. 1. Sampling locations of the Indonesian instant herbal beverages used in this study. B1-B114 represent the sample IDs used in the present study.

2.4. Exposure assessment resulting from the drinking of instants herbal beverages based on methyleugenol and eugenol levels detected

In order to assess the potential exposure to methyleugenol or eugenol resulting from drinking the herbal beverages, the estimated daily intake (EDI) was calculated. The EDI values were expressed in μg/kg bw/day using a body weight (bw) of 54 kg, the average bw for Indonesian male and female (FAO, 2017). For the products targeted at children as their consumers, the EDI was calculated using a bw of 27.21 kg, the average bw of Indonesian boys and girls under the age of 1–17 years old (FAO, 2017).

The EDI calculation was done using the levels detected in the various samples and the recommended daily consumption of these samples as presented on the product labels (Table 1). The weight of the recommended daily consumption (g) was based on the preparation method indicated on the label (See Table 1 at Supplementary materials), assuming that 1 teaspoon equals 6 g, and 1 tablespoon equals 10 g, representing the average of weighting the respective samples using a full tea spoon and tablespoon from Indonesia (3 replications). When there was no information on the label (unknown) the weight and frequency of consumption were assumed to be equal to 3 full teaspoons once a day. In line with the habits for use of the herbal beverages, it was assumed that consumption of the herbal beverage implies consumption of the whole preparation mixed with water. EDI values were calculated following Equation (1):

$$EDI\left(\frac{\mu g/kg\ bw/day}{day}\right) = \frac{recommended\ daily\ consumption\ (g/day) \times level\ of\ detected\ methyleugenol\ (\mu g/g)}{bw\ (kg)} \quad (1)$$

2.5. Benchmark dose (BMD) modelling for methyleugenol

To define the lower confidence limit of the benchmark dose resulting in a 10% extra cancer incidence ($BMDL_{10}$) the quantal dose-response data for induction of hepatocellular carcinoma in male and female F344/N rat induced by methyleugenol and reported by the NTP (2000) (See supplementary Table 2) were used for BMD modelling. In the 2-years study, 50 rats per group for both sexes, were administered methyleugenol orally in 0.5% methylcellulose at doses of 0, 37, 75, or 150 mg/kg, 5 days per week for 105 weeks. For the modelling, these experimental dose levels were converted to the time-adjusted dose levels (mg/kg bw/day) by multiplying the actual dose by 5/7 (to correct for the 5 instead of 7 days per week dosing regimen) in line with what was reported before by Van Den Berg et al. (2011) and Benford et al. (2010).

Previously $BMDL_{10}$ values for methyleugenol were obtained using EPA BMDS software version 2.6.0.1 using different models, including the Gamma, Logistic, Log-logistic, Probit, Log-probit, Multistage, Weibull and Quantal linear model (Van Den Berg et al., 2011). This resulted in values between 15.3 and 34.0 mg/kg bw/day resulting from male and female rat data. In the present study the data were analyzed using model averaging, as recommended by the EFSA-Scientific-Committee et al. (2017), to derive the final $BMDL_{10}$ from multiple fitted benchmark dose models. To this end the online EFSA's web-tool for BMD analysis (https://shiny-efsa.openanalytics.eu/app/bmd), which implements statistical methods for R-package PROAST, version 65.7 Proast (EFSA, 2017) was used. The $BMDL_{10}$ model averaging was performed using the default settings.

2.6. Safety assessment using the Margin of Exposure (MOE) approach for methyleugenol

The MOE approach was applied to assess the risk posed by the use of the methyleugenol containing herbal beverages, in line with the recommendations of EFSA for risk assessment of compounds that are both genotoxic and carcinogenic (EFSA, 2005). The final $BMDL_{10}$ resulting from model averaging using male rat data (providing a lower value than obtained from the female data, see Result section) and EDI values were used to calculate the MOE values according to Equation (2). MOE values were rounded to a single significant figure.

$$MOE = \frac{BMDL_{10}}{EDI} \quad (2)$$

The MOE values are based on chronic lifetime exposure, although realistic use of the herbal drinks may be for shorter periods of time. Although there is no officially established method to evaluate shorter than lifetime exposure to a genotoxic carcinogen, it has been suggested to use Haber's rule to estimate the effects for different exposure duration (Doull and Rozman, 2000; Gaylor, 2000). Haber's rule states that the dose times the effect is constant, (C1 × T1 = constant = C2 × T2) which implies that one could correct for shorter time of exposure in a linear way. Using this approach the MOE values were also calculated for regular short term exposure, i.e. 2 weeks, once every year during a lifetime. This exposure scenario was chosen to mimic the situation where people consume a herbal beverage as a supplement to counteract cold or during periods of illness. In addition, Haber's rule was also used to calculate the number of weeks of daily consumption of the different herbal beverage samples that would result in an MOE value of 10,000, the threshold for risk management concern (EFSA, 2005).

2.7. Safety assessment of eugenol containing herbal beverages

The risk of consuming herbal beverages which contained eugenol was assessed using the Acceptable Daily Intake (ADI) of 0–2.5 mg/kg bw established by JECFA (2006) and the ADI of 1 mg/kg bw/day established by EFSA (2012b), in line with the recommendations of EFSA for risk assessment of compounds that are non-genotoxic (Peña et al., 2017).

2.8. Evaluation of the product registration established in Indonesia based on the safety assessment results and product registration type

To evaluate the product registration as applied in Indonesia during the product registration, this product registration was reviewed based on the results of the safety assessment in the present study. The product registration on the label of 114 samples indicated BPOM RI MD (domestic processed food, 31 samples), BPOM RI ML (foreign processed food, 1 sample), BPOM RI SD (domestic supplement, 9 samples), BPOM RI TR traditional medicine (traditional medicine, 30 samples) and Depkes RI P-IRT samples sold as regular home industry food product (food household industry, 43 samples).

3. Results

3.1. Methyleugenol and eugenol levels in Indonesian instant herbal beverages

Table 1 presents the level of the alkenylbenzenes, methyleugenol and eugenol, as detected and quantified in the herbal beverage samples. Estragole and safrole were not detected. Methyleugenol was detected in 49 out of 114 samples, at levels ranging from 2.6 to 443.7 μg/g. Sample B91, sold as a household industry food product (labelled as P-IRT), appears to contain the highest methyleugenol level at 443.7 μg/g. In 65 of the 114 samples 65 levels of all three alkenylbenzenes were below their respective limit of detection (LOD) of 1.2, 3.0 and 5.5 μM for methyleugenol, safrole and estragole, respectively. Interestingly, 4 out of 114 samples, B5, B19, B83 and B109 appeared to contain eugenol at a level of 21.4–101.2 μg/g.

Table 1
The level of methyleugenol and eugenol detected in the instant herbal beverages and the resulting estimated daily intake (EDI) calculated using the recommendations for daily consumption on the label and assuming the level to equal the LOD of 1.2 μM (1.9 μg/g) for samples where methyleugenol was below the LOD to provide an upper bound EDI.

Sample ID	Detected compound	Level (μg/g)[a]	Recommended daily consumption (g) of the sample	Specified consumer	EDI (μg/kg bw/day)
B1	Methyleugenol	14.0 ± 1.6	20	Adult	5.2
B2	Methyleugenol	94.2 ± 1.4	18	Adult	31.4
B3	nd[b]		11	Adult	0.40 (upper bound)[c]
B4	nd		14	Adult	0.51 (upper bound)[c]
B5	**Eugenol**	**101.2 ± 20.6**	**25**	**Adult**	**46.9**
B6	nd		8	Adult	0.3 (upper bound)[c]
B7	nd		8	Adult	0.3 (upper bound)[c]
B8	nd		8	Adult	0.3 (upper bound)[c]
B9	nd		25	Adult	0.9 (upper bound)[c]
B10	nd		11	Adult	0.4 (upper bound)[c]
B11	nd		5	Adult	0.2 (upper bound)[c]
B12	nd		6.3	Adult	0.2 (upper bound)[c]
B13	nd		21	Adult	0.8 (upper bound)[c]
B14	nd		4	Adult	0.1 (upper bound)[c]
B15	nd		7	Adult	0.3 (upper bound)[c]
B16	nd		10	Adult	0.4 (upper bound)[c]
B17	nd		13.5	Adult	0.5 (upper bound)[c]
B18	nd		12	Adult	0.4 (upper bound)[c]
B19	**Eugenol**	**21.4 ± 3.3**	**12.6**	**Adult**	**5.0**
B20	nd		12.6	Adult	0.5 (upper bound)[c]
B21	nd		30	Adult	1.1 (upper bound)[c]
B22	nd		25	Adult	0.9 (upper bound)[c]
B23	nd		8	Adult	0.3 (upper bound)[c]
B24	Methyleugenol	18.2 ± 4.8	60	Adult	20.3
B25	Methyleugenol	34.3 ± 1.6	20	Adult	12.7
B26	nd		15	Children	1.1 (upper bound)[c]
B27	nd		15	Children	1.1 (upper bound)[c]
B28	nd		21	Children	1.5 (upper bound)[c]
B29	nd		21	Children	1.5 (upper bound)[c]
B30	nd		21	Children	1.5 (upper bound)[c]
B31	nd		21	Children	1.5 (upper bound)[c]
B32	Methyleugenol	110.6 ± 0.5	25	Adult	51.2
B33	nd		15	Children	1.1 (upper bound)[c]
B34	nd		15	Children	1.1 (upper bound)[c]
B35	nd		15	Children	1.1 (upper bound)[c]
B36	nd		15	Children	1.1 (upper bound)[c]
B37	nd		15	Children	1.1 (upper bound)[c]
B38	nd		15	Children	1.1 (upper bound)[c]
B39	nd		15	Children	1.1 (upper bound)[c]
B40	nd		21	Adult	0.8 (upper bound)[c]
B41	nd		8	Adult	0.3 (upper bound)[c]
B42	Methyleugenol	65.06 ± 48.05	23	Adult	27.7
B43	nd		28	Adult	1.0 (upper bound)[c]
B44	nd		25	Adult	0.9 (upper bound)[c]
B45	nd		12.6	Adult	0.5 (upper bound)[c]
B46	nd		25	Adult	0.9 (upper bound)[c]
B47	nd		25	Adult	0.9 (upper bound)[c]
B48	Methyleugenol	16.1 ± 2.5	25	Adult	7.5
B49	Methyleugenol	17.5 ± 6.8	26	Adult	8.4
B50	nd		21	Children	1.5 (upper bound)[c]
B51	nd		25	Adult	0.9 (upper bound)[c]
B52	nd		8	Adult	0.3 (upper bound)[c]
B53	nd		8	Adult	0.3 (upper bound)[c]
B54	nd		8	Adult	0.3 (upper bound)[c]
B55	Methyleugenol	9.6 ± 0.2	22.5	Adult	4.0
B56	Methyleugenol	31.2 ± 0.3	30	Adult	17.3
B57	Methyleugenol	7.1 ± 0.4	25	Adult	3.3
B58	nd		25	Adult	0.9 (upper bound)[c]
B59	Methyleugenol	4.2 ± 0.3	25	Adult	1.9
B60	nd		21	Children	1.5 (upper bound)[c]
B61	nd		21	Adult	0.8 (upper bound)[c]
B62	Methyleugenol	4.2 ± 1.7	21	Children	3.3
B63	nd		30	Adult	1.1 (upper bound)[c]
B64	Methyleugenol	11.9 ± 2.0	25	Adult	5.5
B65	nd		27	Adult	1.0 (upper bound)[c]
B66	nd		25	Adult	0.9 (upper bound)[c]
B67	Methyleugenol	35.9 ± 15.5	15	Adult	10.0
B68	Methyleugenol	28.4 ± 17.5	15	Adult	7.9
B69	nd		15	Adult	0.5 (upper bound)[c]
B70	nd		15	Adult	0.5 (upper bound)[c]
B71	nd		15	Adult	0.5 (upper bound)[c]
B72	nd		10	Adult	0.4 (upper bound)[c]

(*continued on next page*)

Table 1 (*continued*)

Sample ID	Detected compound	Level (μg/g)[a]	Recommended daily consumption (g) of the sample	Specified consumer	EDI (μg/kg bw/day)
B73	Methyleugenol	13.0 ± 0.1	25	Adult	6.0
B74	Methyleugenol	16.1 ± 1.3	10	Adult	3.0
B75	Methyleugenol	51.6 ± 1.5	10	Adult	9.6
B76	Methyleugenol	19.8 ± 1.6	25	Adult	9.2
B77	Methyleugenol	12.7 ± 2.2	30	Adult	7.0
B78	Methyleugenol	11.4 ± 9.1	20	Adult	4.2
B79	Methyleugenol	14.2 ± 2.3	20	Adult	5.3
B80	Methyleugenol	11.4 ± 1.3	18	Adult	3.8
B81	nd		18	Adult	0.7 (upper bound)[c]
B82	Methyleugenol	2.7 ± 1.2	23	Adult	1.1
B83	**Eugenol**	**37.8 ± 6.9**	**25**	**Adult**	**17.5**
B84	Methyleugenol	14.2 ± 0.8	10	Adult	2.6
B85	Methyleugenol	42.6 ± 6.6	33	Adult	26.1
B86	Methyleugenol	66.9 ± 2.9	25	Adult	31.0
B87	Methyleugenol	24.0 ± 3.7	40	Adult	17.8
B88	Methyleugenol	28.7 ± 1.7	22	Adult	11.7
B89	nd		28	Adult	1.0 (upper bound)[c]
B90	nd		75	Adult	2.7 (upper bound)[c]
B91	Methyleugenol	443.7 ± 31.5	6	Adult	49.3
B92	Methyleugenol	10.1 ± 0.4	30	Adult	5.6
B93	Methyleugenol	9.3 ± 0.4	15	Adult	2.6
B94	Methyleugenol	28.9 ± 5.7	15	Adult	8.0
B95	Methyleugenol	36.9 ± 1.2	15	Adult	10.2
B96	Methyleugenol	23.2 ± 1.0	22	Adult	9.5
B97	Methyleugenol	36.3 ± 3.0	23	Adult	15.5
B98	Methyleugenol	24.1 ± 1.3	20	Adult	8.9
B99	Methyleugenol	21.2 ± 1.0	27.5	Adult	10.8
B100	Methyleugenol	2.6 ± 0.3	25	Adult	1.2
B101	Methyleugenol	2.6 ± 1.1	22.5	Adult	1.
B102	Methyleugenol	50.8 ± 3.3	25	Adult	23.5
B103	Methyleugenol	85.0 ± 6.5	20	Adult	31.5
B104	Methyleugenol	109.3 ± 9.5	25	Adult	50.6
B105	Methyleugenol	33.1 ± 0.3	25	Adult	15.3
B106	nd		25	Adult	0.9 (upper bound)[c]
B107	nd		24	Adult	0.9 (upper bound)[c]
B108	Methyleugenol	15.8 ± 4.3	20	Adult	5.9 (upper bound)[c]
B109	**Eugenol**	**26.2 ± 5.7**	**30**	**Adult**	**14.6**
B110	Methyleugenol	17.0 ± 1.4	25	Adult	7.9
B111	Methyleugenol	31.5 ± 1.5	10	Adult	5.8
B112	Methyleugenol	58.4 ± 1.1	20	Adult	21.6
B113	nd		20	Adult	0.7 (upper bound)[c]
B114	nd		25	Adult	0.9 (upper bound)[c]

[a] Mean of 3 independent analyses. Levels were corrected for the recovery result (see materials and methods).
[b] Not detected.
[c] The EDI values of samples in which levels were below the LOD, were calculated using the LOD of methyleugenol of 1.2 μM corresponding to 1.9 μg/g sample thus representing an upper bound.

Table 2
Results from the BMD model averaging to derive a $BMDL_{10}$ for methyleugenol using online EFSA statistical models and the data from the NTP study on hepatocellular carcinoma incidences in methyleugenol-treated male rats exposed via gavage for 2 years (NTP, 2000) (Supplementary Table 2). Fig. 1 (Supplementary materials) presents the corresponding figures.

Fitted Models									Weights for Model Averaging
Model	Number of parameter	Log-likelihood	AIC	Accepted	BMDL	BMDU	BMD	conv	
Null	1	−105.38	212.8		NA	NA	NA	NA	
Full	4	−84.05	176.1		NA	NA	NA	NA	
Two stage	3	−85.06	176.1	Yes	19.3	47.9	35.3	yes	0.08
Log-logistic	3	−84.70	175.4	Yes	21.9	49.8	35.0	yes	0.12
Weibull	3	−84.91	175.8	Yes	20.3	50.4	34.3	yes	0.10
Log-probit	3	−84.50	175	Yes	23.3	49.6	35.7	yes	0.15
Gamma	3	−84.77	175.5	Yes	21.1	50.4	35.2	yes	0.11
Logistic	2	−85.67	175.3	Yes	34.0	48.6	40.8	yes	0.12
Probit	2	−85.38	174.8	Yes	31.6	45.1	37.8	yes	0.07
LVM: Exponential model 3	3	−85.22	176.4	Yes	17.8	50.9	32.6	yes	0.07
LVM: Hill model 3	3	−85.10	176.2	Yes	18.8	50.5	33.2	yes	0.08
Final BMDL/D value					**22.2**	53.7			

AIC (Akaike information criterion); BMD (Benchmark dose); BMDL (lower bound of the BMD confidence interval); BMDU (upper bound of the BMD confidence interval), conv (convergence).

3.2. EDIs of methyleugenol and eugenol resulting from daily consumption of the herbal beverages

Using the quantified levels of methyleugenol and eugenol in the various samples and the direction for their use as indicated on the labels (supplementary materials Table 1), EDI values were calculated using Equation (1). EDI values thus obtained range from 1.1 to 51.2 μg methyleugenol/kg bw/day for the 49 positive samples containing methyleugenol (see Table 1). Sample B32, sold as a traditional medicine (labelled BPOM RI TR), appears to result in the highest EDI, followed by B104 (labelled BPOM RI MD) and B91 (labelled Depkes RI P-IRT) with EDI values amounting to respectively 50.6 and 49.3 μg/kg bw/day. For samples in which levels of methyleugenol were below the LOD, EDI values were calculated by a so-called upper bound approach, assuming levels to be at the LOD of 1.9 μg/g sample. The EDI values thus obtained for these 65 samples in which methyleugenol was below the LOD ranged from 0.1 to 2.7 μg/kg bw/day. This approach to use the LOD to substitute the results below the LOD to define an upper bound for the exposure was recommended by the WHO GEMS/Food EURO workshop (1995). Fifteen out of the 16 samples (94%) specified to be consumed by children appeared to contain no methyleugenol resulting, using the same upper bound approach, in upper bound EDI values of 1.1–1.5 μg/kg bw/day. The EDI of eugenol calculated for the 4 eugenol containing samples (out of 114) ranged from 5.0 to 46.9 μg/kg bw/day.

3.3. $BMDL_{10}$ value for methyleugenol obtained by model averaging

Given that $BMDL_{10}$ values for methyleugenol available in the literature were derived from single model fits in some cases selecting the lowest value, and that at the present state-of-the-art model averaging is considered the preferred method, the $BMDL_{10}$ value for methyleugenol was recalculated using model averaging. Tumor data analyzed were the data from the NTP study reporting dose-dependent incidences of hepatocellular carcinoma in rats exposed to methyleugenol via gavage for 2 years (NTP, 2000) (Supplementary Table 2). The time-adjusted dose levels (mg/kg bw/day) calculated by multiplying the actual dose by 5/7 were used to correct for the 5 instead of 7 days per week dosing regimen (Benford et al., 2010; Van Den Berg et al., 2011). The final $BMDL_{10}$ values resulting from model averaging were 22.2 mg/kg bw/day for male rats (Table 2) and 66.5 mg/kg bw/day for female rats (Table 3 of supplementary materials). Figures representing the model fits are also presented in the Supplementary materials Figs. 1 and 2.

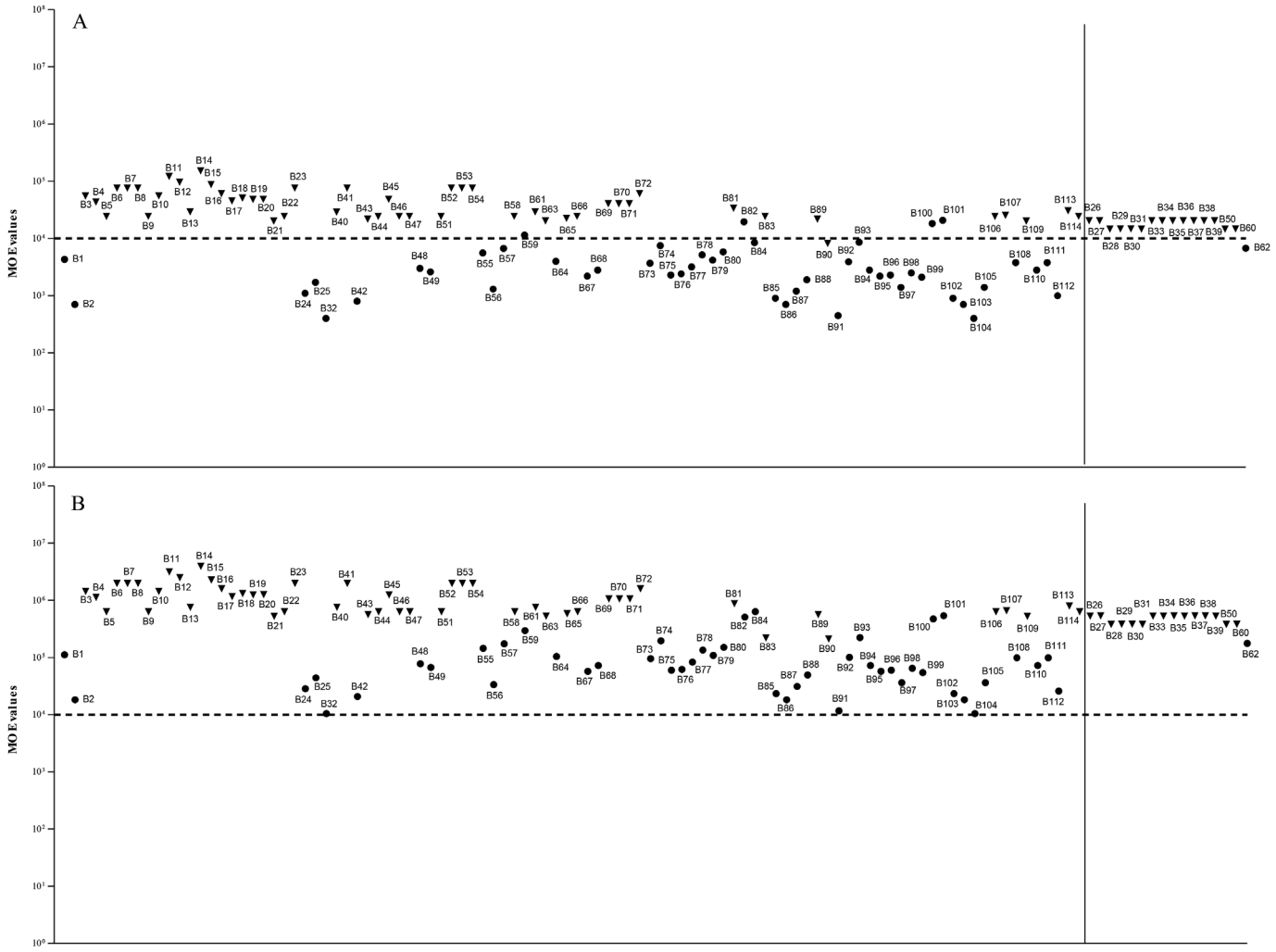

Fig. 2. MOE values obtained for the evaluation of drinking the 114 herbal beverage samples targeted at adults (left of vertical black bar) and children (right side of the black vertical line) based on: A. daily lifetime exposure, B. 2 weeks every year exposure. MOE values were calculated as explained in the Materials and Methods section (equation (2)), using the $BMDL_{10}$ of 22.2 mg/kg bw/day resulting from model averaging (Table 2). The triangles show the MOE values calculated for the samples in which methyleugenol levels were below the LOD and EDI values were calculated as the upper bound (LOD) setting the levels equal to the LOD. The circles represent samples which contained methyleugenol above the LOD. The horizontal dotted line represents the MOE value of 10,000 (——) as a threshold for risk management action (EFSA, 2005).

3.4. MOE based risk assessment for methyleugenol

Considering the target consumer for the herbal beverages samples, the MOE based risk assessment in this study separated into adults and children (Table 1). Fig. 2A presents the MOE values calculated using the $BMDL_{10}$ of 22.2 mg/kg bw/day and the EDIs resulting from regular daily consumption of the 114 samples targeted at adults and children. The results presented in Fig. 2A reveal that for 45 out of the 98 samples (45.9%) of Indonesian instant herbal beverages targeted at adults and 1 out of 16 samples (6.3%) targeted at children the MOE values based on the upper bound EDI were < 10,000 indicating there is a priority for risk management when these herbal beverages would be used every day during a lifetime. For sample B90, which showed analytical results for methyleugenol below the LOD, use of the upper bound level and corresponding EDI resulted in MOE values that amounted to 8200. For the samples targeted at children only 1, B62 in which methyleugenol could be detected and quantified indicated a priority for risk management (MOE < 10,000). Most of the herbal beverage samples which were sold as traditional medicine (labelled BPOM RI TR) to cure trichinosis for children are a low priority for risk management (MOE > 10,000). Of the 49 samples in which methyleugenol could be quantified only 4 samples, B59, B82, B100 and B101, resulted in MOE values > 10,000 indicating a low concern for human health and a low priority for risk management when they would be consumed every day during a lifetime.

Considering that a scenario in which people consume the herbal beverage every day of their life for a whole lifetime seems unrealistic, a risk assessment for other, more realistic exposure scenario's was performed as well. Although there is no formal method to apply the MOE approach for less than lifetime exposures, previous studies have used Haber's rule to correct for shorter than lifetime exposure in a linear way (Doull and Rozman, 2000). In a more realistic scenario people might consume herbal beverages regularly for short periods of time, for example 2 weeks, every year during a lifetime. Following Haber's rule and thus a linear correction of the intake estimates, the EDI values will be 52 weeks per year/2 weeks = 26 times lower and thus the MOE values 26 times higher than what was presented in Fig. 2A adult (98 samples) and children (16 samples) for lifetime exposure scenario's. This resulted in the MOE values depicted in Fig. 2B in which all MOE values are > 10,000 indicating no priority for risk management for all 114 herbal beverages.

In a final assessment it was calculated for the 46 herbal beverage samples that showed MOE values below 10,000 in the initial assessment (Fig. 2A), how many weeks of exposure would result in an MOE value that is 10,000 or higher and thus would not raise a concern. Fig. 3 depicts the results obtained and reveals that the shortest time of daily exposure without raising a concern was for B32 and B104 (the samples with the highest EDIs of 51.2 μg/kg/bw/day and 50.6 μg/kg/bw/day respectively) amounting to 144 weeks equal to about 2 weeks per year during a lifetime. Thus it can be concluded that overall, if the herbal beverages would be consumed for less than 144 weeks (about 2 weeks a year) during a lifetime their level of methyleugenol would not raise a concern.

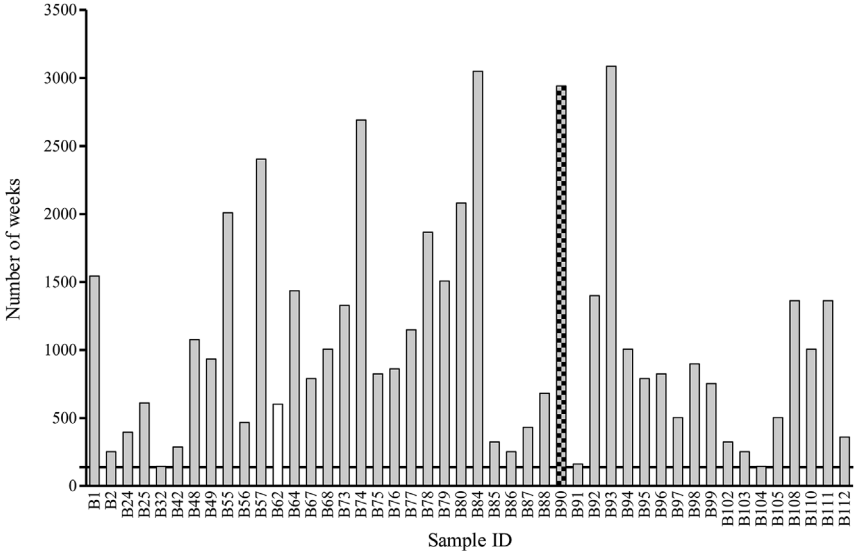

Fig. 3. The number of weeks of exposure that would result in an MOE of 10,000 upon daily consumption of 46 Indonesian herbal beverage samples which showed concern for risk management. White bar represents a sample for children, while the grey patterned bar shows a sample in which methyleugenol levels were below the LOD. The horizontal black line represent 138 weeks, (2 weeks intake a year) during a lifetime.

3.5. Eugenol safety assessment

In 4 of the 114 samples eugenol was detected at a level that could be quantified. Given that eugenol is known to be non-genotoxic its risk assessment can be based on an ADI of 0–2.5 mg/kg bw/day established by JECFA (2006) or the ADI of 1.0 g/kg bw/day established by EFSA (2012b). All of the eugenol containing samples are specified to be consumed by adults. The EDI of eugenol calculated for the 4 eugenol containing samples ranged from 5.0 to 46.9 μg/kg bw/day, far lower than both ADI values, indicating there is no concern.

3.6. Evaluation of the safety assessment result based on the product registration type

Considering the product registration category, it appears that 31 out of the 43 samples (72.1%) produced and labelled as household herbal beverages (labelled Depkes RI P-IRT) appeared to result in MOE values < 10,000 and thus present a possible priority for risk management action upon lifetime consumption (Table 3). Seven out of 31 samples (22.6%) of the herbal beverages categorized as domestic processed food resulted in methyleugenol intakes that did raise a concern for human health. For traditional medicines this number amounted to 7 out of 30 (23.3%). This analysis indicates that product registration may need to include a more detailed safety and/or risk-benefit assessment.

4. Discussion

The aim of the present study was to evaluate whether the level of methyleugenol in instant herbal beverages obtained by a targeted sampling strategy on the Indonesian market would be safe for human consumption. In this targeted sampling strategy samples were collected on the Indonesian market focussing on samples that listed methyleugenol-containing botanicals on their label followed by analysis of their methyleugenol content and resulting EDI and MOE values. Use of the

Table 3
Overview of the risk characterization using the MOE approach for the different Indonesian instant herbal beverages.

Registration code per target consumer	Category of product	Total samples	Concern for risk management	
			Yes	No
Children				
BPOM RI TR	Traditional medicine	16	1	15
Adult				
Depkes RI P-IRT	Food Household Industry	43	31	12
BPOM RI MD	Domestic Processed Food	31	7	24
BPOM RI TR	Traditional medicine	14	6	8
BPOM RI SD	Domestic supplement	9	0	9
BPOM RI ML	Foreign Processed Food	1	1	0
Total		**114**	**46**	**68**

MOE is in line with what is proposed by EFSA and others for risk assessment of compounds in food that are both genotoxic and carcinogenic (Benford et al., 2010; EFSA, 2005; Smith et al., 2010). Since in addition to methyleugenol in some samples also eugenol was detected a risk assessment for eugenol using the ADI of 2.5 mg/kg/day established by JECFA (2006) and the ADI of 1.0 mg/kg bw/day established by EFSA (2012b) was also performed.

Methyleugenol appeared to be the only alkenylbenzenes detected in 49 out of 114 samples of instant herbal beverages. The highest levels were measured in B91 a sample containing robusta coffee and ginger as ingredients. The high occurrence of methyleugenol in the herbal beverages is in line with the fact that most samples contain ginger as a major ingredient, since it was confirmed earlier that ginger (*Zingiber officinale* Rosc) naturally contains this alkenylbenzene (EFSA, 2012a; Tan and Nishida, 2012). Furthermore Singh et al. (2008) reported that methyleugenol was identified at levels amounting to 0.5% of the oleoresin derived from *Z. officinale*. Methyleugenol naturally occurs in other herbs, like nutmeg, cloves, lemongrass, betel pepper and basil (Tan and Nishida, 2012) and the presence of these botanical ingredients in the herbal beverage samples B2, B32 and B104, therefore also likely contributed to the methyleugenol levels detected.

It is of interest to note that the difference in the methyleugenol levels between the different beverages might be more than 100-fold. This relates to 1) the actual level of the methyleugenol containing herb in the sample, and 2) the level of methyleugenol in this botanical. This latter level is known to vary with the part of the plant used, the geographic variants, the growth conditions, physiological variations, evolution and genetic factors, growth stages of the plant (Al-Kateb and Mottram, 2014), maturity of the plant at harvesting, the harvesting techniques, circumstance of storage, processing technologies, and measurement methods (Speijers et al., 2010). On the other hand, the methyleugenol level of 65 out of 114 was below the LOD. Although the results indicate that eugenol was detected in 4 out of 114 samples, the EDI resulting from use of these samples as herbal preparation appeared to be below the ADI of eugenol of 2.5 mg/kg bw/day (JECFA, 2006) indicating this does not raise a concern.

The level of eugenol detected in 4 out of the 114 samples analysed also varied more than 4 fold. The highest level of eugenol was detected in sample B5, sold as a traditional medicine (labelled BPOM RI TR). The relatively high level of eugenol in this sample may be due to the fact that extract of tamarind (*Tamarindus indica* L.) pulp contributed 14% to the ingredients, in addition to extract of betel (*Piper betle* L.) leaf (5.6%) and honey (2.8%). These 3 ingredients have been reported to contain eugenol (Api et al., 2016). Cinnamon and clove, also well known to contain eugenol (Api et al., 2016), likely contributed the eugenol levels in samples B83 and B109, sold as household herbal beverages (both labelled Depkes RI P-IRT). The highest EDI for eugenol registered in the present study of 46.9 μg/kg bw/day (2.5 mg/day for a 54 kg person) resulting from intake of eugenol via herbal beverage sample B5 was lower than the maximum estimated intake in the EU from all sources previously reported to amount to 3 mg/day (EFSA, 2009b). Spices and essential oil are the major contribution to the intake of eugenol in the EU. Given that even this highest intake was below the ADI of 2.5 mg/kg bw/day (JECFA, 2006) and also below the ADI of 1.0 mg/kg bw/day established by EFSA (2012b) also exposure to eugenol via herbal drinks does not raise a concern.

The present study revealed an up to about 48 times difference in the EDI for methyleugenol resulting from consuming the different methyleugenol-containing herbal beverages, a difference that is caused by differences in their methyleugenol levels, but also by differences in the recommended daily use of the samples as indicated on the label, varying from 6 to 60 g per day. The highest EDI of 2765.6 μg methyleugenol/person/day as calculated for B32 based on the EDI of 51.2 μg/kg bw/day and 54 kg bw for Indonesian people (FAO, 2017), appears to be 34.4 fold higher than the estimated per capita intake of methyleugenol of 80.5 μg/person/day from spices and oil for the USA population and 288.1 fold higher than the 9.6 μg/person/day originating from nutmeg, mace and corresponding essential oils estimated for the EU population (William and Mattia, 2009).

Given that exposure to methyleugenol may occur also from other food sources it is of interest to also compare the EDI from herbal beverages estimated in the present study to the EDI for methyleugenol from all sources, estimated to amount to 190 μg/kg bw/day (SCF, 2001) or to 1–10 μg/kg bw/day (Smith et al., 2002).This comparison reveals that intake from herbal drinks may contribute substantially to the dietary intake of methyleugenol. This is especially the case when it is considered that current intake of methyleugenol from the regular diet is likely substantially lower than what was estimated before, because at present addition of methyleugenol as a pure compound to flavour food is no longer allowed (EC, 2008).

Methyleugenol has been associated with carcinogenicity and genotoxicity, in animal studies, although epidemiological data to show the relevance of these effects for the human population are absent. In the absence of human data, risk assessment is based on animal data for tumor formation. In the present study the $BMDL_{10}$ derived from available data on dose-dependent induction of hepatocellular carcinoma in a 2 year rat study (NTP, 2000) was used as a reference point to calculate the Margin of Exposure (MOE). Model averaging as an update on BMD modelling for toxicologically based risk assessment was applied to calculate the $BMDL_{10}$ used. Model averaging is preferred over selecting the lowest $BMDL_{10}$ from results of fitting separate models (EFSA-Scientific-Committee et al., 2017). Model averaging estimates the $BMDL_{10}$ as a weighted average of the outcomes of individual models in which the weight factor is determined by the Akaike Information Criterion (AIC). The AIC represents the goodness of fit of different mathematical models to a dose-response data set (EFSA-Scientific-Committee et al., 2017). The $BMDL_{10}$ value obtained for methyleugenol based on the male rat data by model averaging amounted to 22.2 mg/kg bw/day and appeared to be lower than the value derived from the data for female rats that amounted to 66.5 mg/kg bw/day, reflecting the higher sensitivity of male rats. This value of 22.2 mg/kg bw/day was used to calculate the MOE values. The value falls within the range of $BMDL_{10}$

values of 15.3–34.0 mg/kg bw/day reported before when analysing the rat male data by individual models (Van Den Berg et al., 2011), and is somewhat higher than the lowest $BMDL_{10}$ of 15.3 mg/kg bw used before in risk assessment of methyleugenol containing food supplements or samples of pesto (Al-Malahmeh et al., 2017; Van Den Berg et al., 2011).

Given that an MOE value below 10,000 indicates a possible concern from a public health point of view and points at a priority for risk management actions (EFSA, 2005), the results of the present study indicate that for 46 out of 114 herbal beverages there is a priority for risk management. This risk assessment is however based on lifetime daily consumption of the herbal beverages and one may question whether this is a realistic exposure scenario.

In the absence of a generally established method to take less than lifetime exposure scenario's into account in risk assessment for genotoxic carcinogens by the MOE approach, an estimate of the risk associated to short term use of the herbal beverages was obtained using Haber's rule. Such a shorter period of consumption of the herbal beverages should be taken into account to better reflect the real life exposure scenario in which Indonesian people do not consume these preparations every day during their lifetime. Applying Haber's rule and assuming use for only 2 weeks every year of a lifetime the MOE values were 26 times higher than the MOE values for lifetime exposure, and were all > 10,000 indicating a low priority of risk management. It is of importance to note that Haber's rule can be applied provided there is a linear relationship between tumour incidence and the dose of the carcinogen (Crump et al., 1976). This linearity is assumed and used more often in risk assessment of genotoxic carcinogens. It is used for example when correcting the dosing regimen in 2 year rodent carcinogenicity studies from 5 to 7 days exposure as done in the present and other studies (Benford et al., 2010; Van Den Berg et al., 2011) for the data used for BMD modelling of the NTP carcinogenicity data for methyleugenol. However such linearity in the dose- and time-dependent response behaviour for tumour induction by methyleugenol is not available, although the existing evidence does support linearity in the dose- and time-dependent bioactivation of methyleugenol to its ultimate carcinogenic 1′-sulfooxy metabolite, and for the DNA adduct formation in both cell models or experimental animals exposed to methyleugenol (Al-Subeihi et al., 2012; Gardner et al., 1997).

As depicted in Table 3, most of the herbal beverage samples, namely 31 out of the 43 samples included in the study (72.1%), categorized as household food (labelled Depkes RI P-IRT), raise a concern when people would consume them every day during a lifetime. Also a substantial number of herbal beverage samples registered as domestic processed food, or traditional medicine indicated a concern for human health. The limitation of producers' knowledge related to food safety and low quality control processes for these products may cause the high content of methyleugenol containing herbs in these products. Putri (2018) reported that the house-hold industry in Indonesia can easily get a license to build independent businesses for their economy improvement, while at the same time the limitation of monitoring from NADFC causes their food products to fail to comply with the quality and safety standard. Regulation NADFC 12/2016 Article 6 on Criteria of Possessed Food stipulates that to be registered every processed food should meet 3 criteria's: (1) safety parameters namely the maximum limit of microbial, physical and chemical contamination, (2) quality parameters, including fulfilment of quality requirements in accordance with the existed standards and requirements; and (3) nutritional parameters according to the established requirements. Beside these 3 criteria's the processed food should comply the label requirement, good manufacturing practices and good distribution practices (NADFC, 2016a). The results of the present study suggest that it would be of use that the process and regulation of manufacturing homemade herbal beverages is monitored more closely and may need to be updated in order to reduce the level of methyleugenol.

Currently, the National Agency for Drug and Food Control of Republic of Indonesia (BPOM RI) regulated estragole and safrole as natural food flavouring, with maximum permitted levels (MPLs) of 10 mg/kg for estragole and 0.1 mg/kg for safrole both in ready to drink beverages. The lower MPL for safrole than for estragole is due to consideration of the natural occurrence and use of estragole as a food flavouring (NAFDC, 2016). However MPLs for methyleugenol in herbal beverages in Indonesia have not yet been established. The levels of methyleugenol of 2.6–443.7 μg/g now encountered are higher than 0.1 mg/kg and for several samples even higher than 10 mg/kg, so they would not be in compliance with the MPLs set in Indonesia for related alkenylbenzenes like safrole and estragole. It is of interest to note that, based on the risk assessment provided in the present study, an MPL value of 10 mg/kg would not be low enough to support safe daily consumption of these beverages during a lifetime, when recommended daily use would amount to the highest recommended daily consumption of 75 g, resulting in an MOE of about 1600. At an MPL of 0.1 mg/kg or 1 mg/kg the MOE would amount to 160,000 or 16,000, and use of the herbal preparation would not be of concern. An MPL of 1 mg/g would be in line with what is established by the European Union (EU), EC Regulation 1334/2008 (EC, 2008). An MPL of 10 mg/kg would however support safe consumption when use would be limited to 2 weeks a year during a lifetime. On the other hand reducing the recommended daily consumption of herbal beverages to 0.3 g per day during a lifetime will be safe to meet the MOE value of 10,000 using the highest methyleugenol level of 443.7 μg/g. Obviously the approach to be taken is subject to a risk management decision.

Further evaluations of the present study revealed that with the levels of methyleugenol detected in the samples, their use would not raise a safety concern or priority for risk management if it would be limited to less than 144 weeks (about 2 weeks per year) during a lifetime. This result indicates that risk management of these herbal beverages may focus on providing information on the label for limitation of the consumption duration. Hermanu (2016) reported that the implementation of food safety aspects for many home industry food items in Indonesia is still limited, and the results of the present study provide an examples of how this could be improved.

In conclusion, consumption of methyleugenol-containing herbal beverages can be considered safe when consumed for less than about 2 weeks a year during a lifetime. This conclusion holds for herbal beverages collected by targeted sampling on the Indonesian market. The study does support the establishment of an MPL for methyleugenol in foods and beverages in Indonesia, in line with what has been done for the related alkenylbenzenes estragole and safrole.

Conflicts of interest

The authors declare that there are no conflict of interest regarding this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.02.001.

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