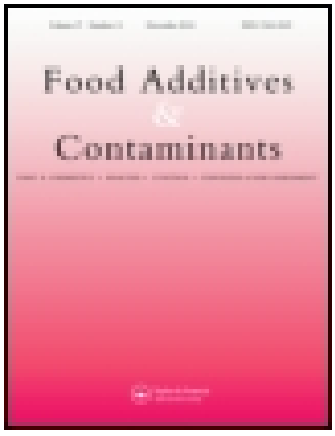

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Herbal food supplements claiming to reduce weight may contain active pharmacological ingredients (APIs) that can be used for the treatment of overweight and obesity. The aim of this study was to determine whether herbal food supplements for weight loss on the Dutch market contain APIs with weight loss properties. Herbal food supplements intended for weight loss (n = 50) were sampled from August 2004 to May 2013. An HPLC-DAD-MS/MS method was used to screen for the presence of the APIs in herbal supplements. In 24 samples the APIs sibutramine, desmethylsibutramine (DMS), didesmethylsibutramine (DDMS), rimonabant, sildenafil and/or the laxative phenolphthalein were identified 41 times. The presence of these APIs was, however, not stated on the label. The potential pharmacological effects of the detected APIs were estimated using data from reported effective doses of approved drugs. Use of 20 of the 24 herbal food supplements may result in potential pharmacological effects. Furthermore, risk assessment of phenolphthalein, a suspected carcinogen and found to be present in 10 supplements, based on the margin of exposure (MOE) approach, resulted in MOE values of 96–30 000. MOE values lower than 10 000 (96–220) were calculated for the daily intake levels of four out of these 10 supplements in which phenolphthalein was found. However, taking into account that weight loss preparations may be used for only a few weeks or months rather than during a lifetime, MOE values may be two to three orders of magnitude higher. The current study shows that the use of food supplements with sibutramine, DMS, DDMS and/or phenolphthalein could result in pharmacological effects.

Keywords: (di)desmethylsibutramine; herbal food supplements; phenolphthalein; rimonabant; sibutramine

Introduction

Nowadays, overweight and obesity are a growing public health issue. The WHO estimates that more than 1.4 billion adults are overweight and at least 500 million people are obese. Overweight can be defined by a body mass index (BMI) between 25 and 30 kg m^{-2}, and obesity is defined by a BMI greater than or equal to 30 kg m^{-2} (WHO 2013).

In the last decennia synthetic drugs for the treatment of overweight and obesity have been introduced onto the market such as Reductil®, Meridia®, Reduxade® and Zelium®, containing sibutramine as the active pharmacological ingredient (API), Acomplia®, containing rimonabant, Fentrate Retard® and Ponderal®, containing fenfluramine, Isomeride®, containing dexfenfluramine, and Xenical®, containing orlistat (Ioannides-Demos et al. 2006; RIVM 2009; EMA 2010a; EC 2013).

Herbal food supplements that claim to induce weight loss are marketed worldwide and are readily available over the Internet (Jordan & Haywood 2007; De Carvalho et al. 2011; Ancuceanu et al. 2013; Ozdemir et al. 2013). These products generally claim to be 'all natural', but there are frequent reports of adulterations with drugs for the treatment of overweight, obesity and constipation such as sibutramine, fenfluramine, rimonabant, orlistat and phenolphthalein (Yuen et al. 2007; Zou et al. 2007; Wang et al. 2008; RIVM 2009; Chen et al. 2009; Vaysse et al. 2010; Dunn et al. 2011; Stypułkowska et al. 2011; Tang et al. 2011; De Carvalho et al. 2012; Phattanawasin et al. 2012; Ancuceanu et al. 2013).

The API sibutramine (Figure 1a), which was initially developed as an antidepressant, is a combined serotonin (5-HT) and noradrenaline (NA) reuptake inhibitor. In the body sibutramine is rapidly metabolised through demethylation into desmethylsibutramine (DMS) (Figure 1b) and didesmethylsibutramine (DDMS) (Figure 1c) (Nisoli & Carruba 2000; Kang et al. 2010a). DMS and DDMS are both pharmacologically active, inducing satiety and stimulation of thermogenesis (Glick et al. 2000; Nisoli & Carruba 2000; Ding et al. 2003; Padwal & Majumdar 2007). In 2010, the European Medicines Agency (EMA) recommended the suspension of market authorisations for

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sibutramine in the European Union because of an increased risk of serious, non-fatal cardiovascular events, such as stroke or heart attack (EMA 2010a). Reported side-effects of sibutramine include cardiovascular effects such as tachycardia and (arterial) hypertension (Ioannides-Demos et al. 2006; Padwal & Majumdar 2007; Müller et al. 2009; Cohen & Ernst 2010). Furthermore, several psychiatric symptoms are reported such as psychosis and (hypo)mania, occurring at dose levels of sibutramine in an estimated range of 2.8–60 mg (Taflinski & Chojnacka 2000; Litvan & Alcoverro-Fortuny 2007; Lee et al. 2008; Müller et al. 2009; Chen et al. 2010; Chong 2010; van Hunsel & van Grootheest 2011; Waszkiewicz et al. 2012).

Also adverse effects of DMS and DDMS have been clearly documented. Three cases of psychotic symptoms were linked to the use of slimming products containing DMS (Yuen et al. 2007; Chen et al. 2010), and cardiovascular effects were reported in a man who had used a herbal weight loss supplement containing DDMS (Fil et al. 2011).

Another weight loss drug, Acomplia®, which contains the API rimonabant (Figure 1d), was withdrawn from the European market at the end of 2008 because of severe side-effects, such as depression and suicidal behaviour, and lack of efficacy (EMA 2007, 2009; Venhuis et al. 2011; EC 2013). Rimonabant is a selective cannabinoid-1 receptor (CB1) blocker that regulates food intake (Van Gaal et al. 2005; Padwal & Majumdar 2007). After the withdrawal of Acomplia® in 2008, counterfeit Acomplia® and imitation products containing rimonabant polymorphs were reported to be available on the Internet (Venhuis et al. 2011).

Phenolphthalein (Figure 1e), which is a benzofuran derivate, was used in a number of authorised medicinal products for treatment of constipation (IARC 2000; NTP 2011). In 1996, the US National Toxicology Program (NTP) published data on the genotoxicity of phenolphthalein and its carcinogenicity in laboratory animal studies (NTP 1996). In 1997, the EMA concluded that the National Competent Authorities should take these NTP data into account in their considerations of any restriction of phenolphthalein containing medical products on the national markets (EMA 1997).

In the Netherlands, the presence of sibutramine and phenolphthalein in a vitamin supplement and in capsules was first reported by the National Customs Laboratory in 2004 (RIVM 2009). Subsequently the Netherlands Food and Consumer Product Safety Authority (NVWA) monitored the presence of APIs in various herbal food supplements for weight loss on the Dutch market. The current study describes the results of this survey, and also evaluates the possible pharmacological relevance of dose levels detected based on data from reported effective doses of approved drugs. Furthermore, in the present study the risk assessment of the suspected carcinogenic effects of phenolphthalein was performed using the margin of exposure (MOE) approach (EFSA 2005). To this end, the MOE was determined by calculating the benchmark dose (BMD) lower confidence limit for 10% extra cancer risk ($BMDL_{10}$) based on tumour data from animal studies and comparing this value with the intakes that would result from daily use of the herbal supplements at the recommended dosing.

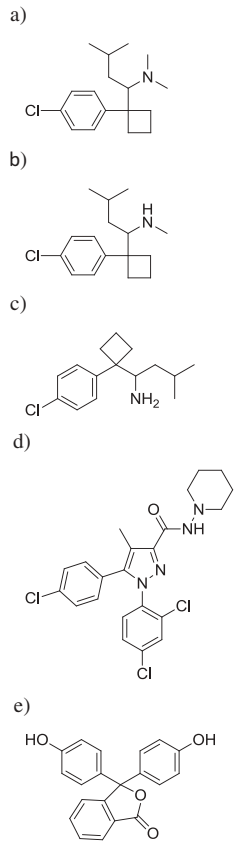

Figure 1. Chemical structures of (a) sibutramine, (b) desmethylsibutramine, (c) didesmethylsibutramine, (d) rimonabant and (e) phenolphthalein.

Materials and methods

Sampling

For the present study, 50 herbal supplements for weight loss were sampled on the Dutch market by NVWA inspectors from August 2004 up to and including May 2013 as a part of normal inspectorate routine. These herbal supplements were sampled in 2004 (n = 5), 2010 (n = 5), 2011 (n = 16), 2012 (n = 21), and 2013 (n = 3). Sampling locations were identified by an Internet search or selected from the NVWA inspection database. The sampling strategy was partly risk based. Inspectors collected products based on experience and on the basis of reports and alerts

of national and international health authorities concerning adulterated food supplements on the market and reports of side-effects linked to specific products. These national and international reports and alerts included an official warning in the Netherlands of side-effects of a food supplement containing green coffee (IGZ 2010) and consumer warnings from health authorities such as from Australia (Australian Government 2007), Canada (Health Canada 2006), the UK (MHRA 2010), and the United States (FDA 2013). These reports and alerts pointed to herbal supplements that might contain APIs and these were indicated for sampling in our sampling strategy. Furthermore, the sampling strategy was also based on reports of side-effects that were directly reported by consumers to the NVWA and on reports of side-effects that were received from the Netherlands Pharmacovigilance Centre (Lareb 2013). Moreover, previous analyses by the Dutch Customs Laboratory of food supplements imported into the Netherlands and findings of the APIs in these products were also taken as a basis for the sampling strategy. Additionally, one of the indicators for identifying suspected herbal supplements was user reports on Dutch Internet forums in which users reported side-effects resulting from the use of herbal slimming products.

The sampled supplements consisted mainly of capsules, tablets and sachets with powder. All samples of herbal supplements were in pre-packaged form and contained instructions for use. Brand names were made anonymous by replacement with roman numerals.

Chromatographic screening for APIs in the herbal supplements

The suspected samples were analysed for pharmacologically relevant levels of APIs immediately after receiving them. In the course of time, several different analysis methods (all based on LC with UV and/or MS-based identification and quantification) were employed depending on availability at our laboratory at the time of analysis. In all cases method validation was carried out in accordance with the general criteria of the European Pharmacopoeia and using reference standards. The fact that low amounts of APIs could be identified indicated the techniques that were adequate for the purpose. For the currently used HPLC-DAD-MS/MS method, half a dose unit was sonicated with methanol and then diluted 100 times using MeOH/0.1% formic acid (buffered at pH 4 with 35% concentrated ammonia). After filtration, over a 0.45 µm filter (Whatman GmbH, Dassel, Germany) the solution was analysed for common weight-loss drugs by LC-DAD-MS/MS. After each sample a blank was injected to prevent carryover. Compounds of interest were found by searching for MH^+ ions (full scan) and marker fragments (MS/MS) (Venhuis et al. 2011, 2014). Quantitation was performed based on the UV response using three- or five-point calibration curves. Because the sample matrices were complex and highly variable, each negative screening result was challenged by spiking the sample with a pharmacologically irrelevant level of sibutramine. In most instances spiked sibutramine could be identified at a level of 0.1 mg $dose^{-1}$. In a few cases the matrix required spiking at a level of up to 0.6 mg $dose^{-1}$ before sibutramine could be identified.

Assessment of the pharmacological effects of the APIs identified in the herbal supplements

In order to investigate whether the use of an analysed herbal supplement would be of pharmacological relevance, the pharmacological potencies and doses of the APIs identified in the herbal supplements were assessed. The pharmacological potency of the APIs found in the sample was estimated by comparing the daily intake of the API that would result from its use according to the manufacturer's prescriptions to the dose information for registered drugs. Furthermore, the recommended daily dose of the herbal supplement was compared with the lowest commercially available daily dose of a drug containing the API. The recommended daily dose of the herbal supplement was identified from the label, or from an Internet search. For the calculation of the resulting intake of the API we selected the upper range of the recommended daily dose of the herbal supplement. For the herbal supplements for which no recommended daily dose could be identified, we assumed the recommended daily dose to be 1 dosing unit day^{-1}. The pharmacological potencies of DMS and DDMS were estimated by comparing data from (patent) literature on pharmacological profiles of sibutramine to DMS and DDMS.

Risk assessment of phenolphthalein identified in the herbal supplements

For the risk assessment of phenolphthalein detected in the herbal supplements, we applied the MOE approach (EFSA 2005). Benchmark dose (BMD) and $BMDL_{10}$ values were obtained by fitting the carcinogenicity data obtained from the literature to a number of different mathematical models using BMDS software, version EPA 2.4 (EPA 2013). The following models were used: Gamma, Logistic, LogLogistic, LogProbit, Multistage Cancer, Multistage, Probit, Quantal-Linear and the Weibull model. BMDS software was applied without model restrictions, using a default setting of an extra risk type, a 95% confidence level and a benchmark response (BMR) of 10%. The $BMD(L)_{10}$ values derived from the different fitted models were only accepted if the fit of the selected model was not of poorer quality than that of the so-called full model representing a perfect fit to the dose–response data. For this purpose, the *p*-value was taken into account with a value below 0.05 resulting in model rejection. For calculation of the MOE, the lowest $BMDL_{10}$ value was selected

(EFSA 2005). The estimated daily intake (EDI) for phenolphthalein from the herbal supplements was calculated based on the concentration of phenolphthalein detected in the supplement and the recommended daily dose indicated on the label, using a default body weight of 70 kg (EFSA 2012). The MOE values were calculated by dividing the $BMDL_{10}$ by the EDI (EFSA 2005).

Results

Presence of APIs in the herbal supplements

In total 50 herbal supplements for weight loss were analysed for the presence of APIs. Table 1 shows the APIs identified, the recommended daily dose in dose units day^{-1} for the respective herbal supplements and the dose levels of the APIs that would result from this level of use. Sibutramine, DMS, DDMS, phenolphthalein, sildenafil or rimonabant were identified in 24 herbal supplements with 12 different brand names. In 11 herbal supplements more than one API was detected. Sibutramine was found in 17 herbal supplements and was the most frequently detected API. In addition, phenolphthalein, DDMS and DMS were found in 10, six and four herbal supplements, respectively. Rimonabant was found in one herbal supplement. Additionally, in two herbal supplements (XVIII and XIX) trace levels (< 0.3 mg dosing $unit^{-1}$) of the PDE-5 inhibitor sildenafil were found, and in one herbal supplement (XVI) a dose level of 0.9 mg dosing $unit^{-1}$ of sildenafil was found (Table 1). The APIs fenfluramine and N-nitrosofenfluramine were not found.

Assessment of the pharmacological effects of the APIs identified in the herbal supplements

The text below discusses for each API identified in the current study whether the use of the herbal supplements that contained the APIs identified would be of pharmacological relevance.

Table 1. Dose levels of the active pharmacological ingredients (APIs) sibutramine (sib), desmethylsibutramine (DMS), didesmethylsibutramine (DDMS), rimonabant (rim), sildenafil (sil) and phenolphthalein (phen) in herbal supplements sampled from the Dutch market.

Herbal supplement[a]	Brand name[b]	Year of sampling	Analytically determined concentration (mg dosing $unit^{-1}$)[c,d]	Recommended daily dose (dosing unit day^{-1})	Estimated daily dose level (mg day^{-1})[e]
I	A	2010	11 (sib)	1	11 (sib)
II	A	2010	21(sib)	1	21 (sib)
III	A	2010	21 (sib)	1	21 (sib)
IV	A	2010	19 (sib)	1	19 (sib)
V	B	2011	18 (sib)	1–2	18–36 (sib)
VI	B	2011	3.7 (DDMS); 0.3 (phen)	1–2	3.7–7.4 (DDMS); 0.3–0.6 (phen)
VII	B	2011	3.6 (DDMS); 0.2 (phen)	1–2	3.6–7.2 (DDMS); 0.2–0.4 (phen)
VIII	B	2011	3.7 (DDMS); 0.2 (phen)	1–2	3.7–7.4 (DDMS); 0.2–0.4 (phen)
IX	B	2011	8 (sib)	1–2	8–16 (sib)
X	C	2011	2 (sib)	2	4 (sib)
XI	C	2011	13 (sib)	2	26 (sib)
XII	D	2011	16.8 (sib)	1	16.8 (sib)
XIII	E	2011	6 (sib); 43 (phen)	1	6 (sib); 43 (phen)
XIV	F	2012	16 (DDMS)	0.5–1	8–16 (DDMS)
XV	F	2012	16 (DDMS)	0.5–1	8–16 (DDMS)
XVI	G	2012	< 0.1 (DDMS); 43 (phen); 0.9 (sil)	1[f]	43 (phen); 0.9 (sil)
XVII	H	2012	12 (sib); 27 (phen)	1	12 (sib); 27 (phen)
XVIII	I	2012	10 (sib); < 0.1 (DMS); < 0.3 (sil)	1[f]	10 (sib)
XIX	J	2012	15 (sib); 31(phen); < 0.3 (sil)	1–2	15–30 (sib); 31–62 (phen)
XX	K	2012	20 (sib)	1[f]	20 (sib)
XXI	A	2012	0.3 (rim)	1[f]	0.3 (rim)
XXII	A	2013	< 0.1 (sib); 0.1 (DMS); < 0.1 (phen)	1[f]	0.1 (DMS)
XXIII	A	2013	< 0.1 (sib); 0.2 (DMS); < 0.1 (phen)	1[f]	0.2 (DMS)
XXIV	L	2013	< 0.1 (sib); 0.1 (DMS); < 0.1 (phen)	1[f]	0.1 (DMS)

Notes: [a]The samples of the herbal supplements are indicated by roman numerals.
[b]The brand names of the herbal supplements are replaced by letters.
[c]Sibutramine, DMS and DDMS are reported as free bases.
[d]Standard deviation (SD) ≤ 20%.
[e]Analysed dose level multiplied by the recommended daily dose.
[f]No recommended daily dose could be identified; the recommended daily dose was set as 1 dosing unit day^{-1}.

Sibutramine

Sibutramine was found in 14 samples at levels that would result in estimated daily dose levels of 4–36 mg sibutramine (as a free base) day^{-1}. Additionally, in three herbal supplements traces of sibutramine (< 0.1 mg dosing unit^{-1}) were found (Table 1). For treatment of weight loss sibutramine-HCl is commercially available as a registered drug in doses of 10 and 15 mg, and prescribed at 1 dosing unit day^{-1} (EMA 2002, 2010b). To estimate the potential pharmacological effect of the herbal supplements, we selected the lowest commercially available dose of 10 mg sibutramine-HCl, which corresponds to 8.3 mg of the free base. The use of 12 herbal supplements (I–V, IX, XI, XII, XVII–XX) at the recommended daily dosing would exceed the lowest commercially available daily recommended sibutramine dose as the free base (8.3 mg) of this formerly registered drug, and these 12 herbal supplements were thus considered to induce pharmacological effects.

DMS

DMS was found to be present in three samples at levels that would result in estimated daily dose levels of 0.1 (XXII, XXIV) and 0.2 mg day^{-1} (XXIII) when used as recommended. Additionally, one herbal supplement contained a small amount of DMS (Table 1). From a literature search we concluded that DMS and DDMS have similar pharmacological effects as sibutramine *in vivo* (Jerussi et al. 2001; Kang et al. 2010b). Furthermore, based on the differences of sibutramine, DDMS and DMS on the uptake of norepinephrine, serotonin and dopamine, we estimated DMS and DDMS to be up to 50-fold more potent than sibutramine (Glick et al. 2000). Patent literature suggests an effective dose for DDMS as off 0.2 mg day^{-1} (Barberich 2005) in the treatment of sleeping disorders. Because of the similarities in the pharmacological profiles of DDMS and DMS, we assume both APIs are pharmacological active at a dose ≥ 0.2 mg day^{-1}. Because the highest estimated daily dose of DMS was 0.2 mg day^{-1} (XXIII), we considered that use of this supplement is likely to induce pharmacological effects.

DDMS

DDMS was found to be present in five samples (VI–VIII, XIV and XV) at levels that, when used as recommended, would result in an estimated intake of 3.6–16 mg day^{-1}. Additionally, one herbal supplement (XVIII) contained a small amount of DDMS (Table 1). Because the lowest estimated daily dose of this API with herbal supplement VII (3.6 mg day^{-1}) is above the lowest dose of DDMS (0.2 mg day^{-1}) of which pharmacological effects are expected, the use of these five supplements as recommended at estimated daily DDMS levels from 3.6 to 16 mg day^{-1} was considered to induce pharmacological effects.

Rimonabant

Rimonabant was found in one herbal supplement (XXI) at a level that would result in an estimated daily dose level of 0.3 mg day^{-1} when used as recommended. The drug Acomplia® is commercially available in doses of 20 mg rimonabant per dosing unit, taken once daily (EMA 2013). Because the estimated daily dose level of rimonabant in herbal supplement XXI appears to be far below the commercially available rimonabant dose of a registered drug amounting to 20 mg rimonabant day^{-1}, it can be concluded that use of this herbal supplement as recommended is unlikely to result in pharmacological effects.

Sildenafil

Sildenafil, which is a phosphodiesterase type 5 inhibitor, was identified on the Dutch market in herbal food supplements used to enhance sexual potency (Reeuwijk et al. 2013). For sildenafil the commercially available dose of a registered drug for the treatment of pulmonary hypertension is 20 mg (Revatio®) and the lowest available dose of sildenafil with a registered drug for the treatment of erectile dysfunction is 25 mg (Viagra®) (Reeuwijk et al. 2013), which is higher than the analysed dose levels of supplements XVI (0.9 mg day^{-1}), XVIII (< 0.3 mg dosing unit^{-1}), and XIX (< 0.3 mg dosing unit^{-1}) (Table 1). It was therefore concluded that use of these three herbal supplements as recommended is not expected to produce pharmacological effects.

Phenolphthalein

Phenolphthalein was found in seven herbal supplements at levels that would result in estimated daily dose levels ranging from 0.2 to 62 mg day^{-1} (Table 1). EMA (1997) reported recommended clinical oral doses of phenolphthalein to be 50–200 mg, and an anticipated human intake in normal users of 4 mg phenolphthalein kg bw^{-1} day^{-1}, which is equivalent to 280 mg day^{-1} for a 70 kg adult. Furthermore, NTP (2011) reported oral dose levels of phenolphthalein available as over-the-counter drugs ranging from 30 to 200 mg for adults. Doses for children, aged from 2–11 years, were reported to be 15–60 mg (NTP 2011). Additionally, IARC (2000) reported phenolphthalein to be available as tablets in a range from 6.5 to 200 mg. For the assessment of the pharmacological effect of phenolphthalein present in the herbal supplements, we assumed the lowest daily dose level of phenolphthalein to be 6.5 mg phenolphthalein day^{-1}, which was reported by the IARC (2000). Estimated daily dose levels of four herbal supplements (XIII, XVI, XVII and XIX) exceeded this selected lowest reported available daily dose, and it was therefore considered that use of these herbal supplements can produce a pharmacological effect.

Altogether, from the assessment of the pharmacological effects of the APIs identified in the herbal supplements, it was concluded that use of 20 out of 24 herbal supplements, in which the APIs sibutramine, DMS, DDMS and/or phenolphthalein were identified, could result in pharmacological effects.

Risk assessment of phenolphthalein identified in the herbal supplements

Aside from its pharmacological effects, phenolphthalein also possesses carcinogenic potency (NTP 1996, 2011; IARC 2000). For the risk assessment of phenolphthalein, we used data from the studies of Dunnick and Hailey (1996) and NTP (1996) on the incidence of hystiocytic sarcomas in $B6C3F_1$ male mice exposed in four dose groups to phenolphthalein for 104 weeks by feed (Table 2). Table 3 shows the results from the BMD analysis of the data from Dunnick and Hailey (1996) and NTP (1996). $BMDL_{10}$ values were calculated and these were in a range of 85–557 mg kg bw^{-1} day^{-1}. For the calculation of the MOE values resulting from exposure to phenolphthalein in herbal supplements, we used the lowest $BMDL_{10}$ value, which was 85 mg kg bw^{-1} day^{-1}. The MOE values obtained using the estimated daily intakes (EDI) that would result from use of the phenolphthalein-containing herbal supplements varied from 96 to 30 000. Use as recommended of four of the herbal supplements containing phenolphthalein (XIII, XVI, XVII and XIX) would result in MOE values ranging from 96 to 220.

It must be emphasised that in this approach MOEs are calculated assuming lifetime exposure while herbal food supplements for weight loss may only be used during relatively short periods for several weeks or months. For this reason, calculation of the MOEs assuming lifetime exposure at the estimated daily intakes might overestimate the potential risk for human health. However, a general framework for taking intermittent and/or short-term instead of lifetime exposures to compounds that are both genotoxic and carcinogenic into account in the safety assessment is currently not in place. Felter et al. (2011) recently proposed using the principle of Haber's Rule to assess the risk from less-than-lifetime exposures to carcinogens, provided that chemical-specific carcinogenicity data are available and that data support a linear dose–response relationship (Felter et al. 2011). Haber's Rule assumes that the acceptable cumulative lifetime exposure can be averaged over the duration of short-term exposure, suggesting that higher daily intakes are acceptable when short-term exposure is considered (Felter et al. 2011). Applying Haber's Rule to assess the potential risk for short-term exposure during a period of several weeks or months on an estimated life expectancy of 75 years may result in MOE values that are two to three orders of magnitude higher than those obtained when assuming lifetime (75 years) daily use of the herbal supplements.

Table 2. Data on the incidence of hystiocytic sarcoma in $B6C3F_1$ male mice exposed to increasing doses of phenolphthalein for 104 weeks by feed (Dunnick & Hailey 1996; NTP 1996) used in the current study for the BMD analysis.

Dose (mg kg bw^{-1} day^{-1})	Number of animals	Incidence of hystiocytic sarcoma
0	50	1
300	50	3
600	50	11
1200	49	12

Discussion

In this study 50 herbal supplements for weight loss available on the Dutch market were investigated for the presence of APIs. In 24 samples the APIs sibutramine, DMS, DDMS, rimonabant, sildenafil and/or the laxative phenolphthalein were identified 41 times, and in 11 herbal supplements more than one API was identified. Possible pharmacological effects, and for phenolphthalein also toxicological effects, resulting from the presence of these APIs in the herbal supplements were evaluated. It was found that the use as recommended of 20 out of the 24 herbal supplements, in which sibutramine, DMS, DDMS and phenolphthalein were identified, would result in estimated daily dose levels that could result in pharmacological effects. Furthermore, the use of four out of 10 herbal supplements containing phenolphthalein would result in daily intake levels that result in MOE values in a range of 96–220, being lower than 10 000. For short term-exposure (a few weeks or months) MOE values may be two or three orders of magnitude higher. The NVWA has taken action such as to fine the suppliers of herbal supplements containing hidden APIs. Furthermore, consumers have been warned not to use such herbal supplements via press releases.

Estimation of pharmacological effective doses of the APIs found

To investigate whether medicinal products legislation could be applied to the sampled herbal supplements, we assessed whether their use would produce a pharmacological effect. For this, we compared the dose level of APIs resulting from the recommended use of the herbal supplements with the lowest dose level of commercially available drugs containing sibutramine, rimonabant, sildenafil and phenolphthalein identified in the literature. We concluded that when the lowest dose level of the commercially available drugs was exceeded, pharmacological effects could be expected. However, it cannot be excluded

that dose levels resulting from the use of herbal supplements at estimated daily dose levels that are below the lowest commercially available daily dose will also produce pharmacological effects. Thus estimates presented for the possible pharmacological effects from the herbal supplements containing these APIs may be underestimates.

A limitation of this study in this respect is that for the APIs DMS and DDMS no commercially available doses exist or have existed. Furthermore, reliable data from literature on the pharmacologically active doses of DMS and DDMS are lacking. Relative to sibutramine, we estimated that DMS and DDMS could produce pharmacological effects at doses that are 50-fold lower (Glick et al. 2000). Based on this estimate we assumed the lowest pharmacologically effective dose of DMS and DDMS to be 50-fold lower than the lowest pharmacologically effective dose of sibutramine (8.3 mg day^{-1}), thus equating to 0.2 mg day^{-1}. It should be noted that 0.2 mg day^{-1} is an estimation of the pharmacological effective dose of DMS and DDMS, and this dose may be an underestimation or an overestimation.

Herbal supplements containing a combination of sibutramine, DMS or DDMS with phenolphthalein

In this study the combination of sibutramine, DMS or DDMS with phenolphthalein was found in 10 herbal supplements. The presence of the combination of sibutramine with phenolphthalein in a herbal supplement was reported earlier in the Netherlands (RIVM 2009). Also in other countries such as the United States (Dunn et al. 2011), France (Vaysse et al. 2010), China (Wang et al. 2008), and Hong Kong (Yuen et al. 2007) combinations of sibutramine, DMS or DDMS with phenolphthalein were reported. Laxatives, like phenolphthalein, are considered to be not very effective as weight loss drugs (Martin et al. 1998). However, a reported side-effect of sibutramine is constipation (Nisoli & Carruba 2000; Wilfley et al. 2007; Müller et al. 2009), and phenolphthalein might have been added because of its laxative properties.

Levels of sibutramine found in herbal supplements with an identical brand name

In this study we identified sibutramine in samples with a similar brand name which varied from trace amounts to levels that would result in an estimated daily dose of 21 mg sibutramine in the herbal supplements I–IV, XXII and XXIII (Table 1). Furthermore, in a sample of a herbal supplement with the same brand name (XXI), sibutramine could not be identified. Moreover, we reported that in two brands of adulterated dietary supplements used for weight loss, the dose units in one package contained either no API, one API or different APIs such as sibutramine, DMS and DDMS, and at different dosages (Venhuis et al. 2013). These results indicate variability in the contamination of herbal supplements of a specific brand name suggesting the APIs are not present at a systematic or fixed level. This suggests that the risks associated with the use of these herbal supplements will be variable because of differences in the levels and type of APIs present. In order to quantify these differences further, future work could focus on analysis of composite samples accompanied by analyses of several samples from individual dose units in one package and from different packages from a specific brand.

Trace levels of APIs

Low levels of sibutramine, sildenafil and phenolphthalein were found in some herbal supplements (XVIII, XIX, XXII, XXIII and XXIV), and low levels of DMS were also found in one product (XVIII). These low levels are not expected to be pharmacologically relevant. Furthermore, the presence of low levels of APIs is most likely a quality problem associated with manufacturing deficiencies such as inadequate cleaning of the equipment between production runs, or inhomogeneous mixing of the API and the herbal matrix resulting in a variable product composition. It is of importance to note that low levels of APIs, in combination with other APIs present at higher levels, might add to the pharmacological effect of the supplement. This possible additive pharmacological effect of combinations of different APIs remains to be investigated.

Sampling protocol

In this study we applied a targeted sampling protocol. Inspectors sampled products based on reports of national and international health authorities concerning adulterated food supplements on the market, and reported side-effects linked to specific products. Prior to analysis we searched the Internet for more information on the sampled food supplements in order to assess the likelihood of APIs being present. In several cases we found in Internet forums on losing weight reports of consumers on side-effects of the sampled products that were later analytically shown to contain APIs. These consumer reports might be used as a crude indicator for the presence of APIs.

Risks phenolphthalein

For the risk assessment of phenolphthalein, which is expected to be a possible human carcinogen (IARC 2000), we calculated $BMDL_{10}$ values that were based on the incidence of hystiocytic sarcomas in $B6C3F_1$ male mice reported by Dunnick and Hailey (1996) and NTP (1996) (Table 3). Although we are aware that this study was based on a limited amount of dosing groups, we decided to use

Table 3. Results from a BMD analysis of the data on the incidence of hystiocytic sarcoma in $B6C3F_1$ male mice exposed to phenolphthalein (Dunnick & Hailey 1996; NTP 1996) using BMDS software version 2.4, a BMD of 10% extra risk and default settings.

Model	Restriction	Number of parameters	Log-likelihood	*p*-value	Accepted[a]	BMD_{10} (mg kg bw^{-1} day^{-1})	$BMDL_{10}$ (mg kg bw^{-1} day^{-1})
Null		1	−79.011	–	–	–	–
Full		4	−69.872	–	–	–	–
Gamma	None	3	−70.989	0.14	Yes	403	85
Logistic	n.a.[b]	2	−72.501	0.07	Yes	690	557
LogLogistic	None	3	−70.935	0.15	Yes	401	98
LogProbit	None	3	−70.838	0.17	Yes	408	117
Multistage Cancer	n.a.[b]	2	−70.993	0.33	Yes	415	292
Multistage	None	3	−70.902	0.15	Yes	357	185
Probit	n.a.[b]	2	−72.227	0.10	Yes	648	519
Quantal-Linear	n.a.[b]	2	−70.993	0.33	Yes	415	292
Weibull	None	3	−70.985	0.14	Yes	398	89

Notes: [a]The fitted model was not significantly different (worse) than the full model at $p < 0.05$.
[b]Not applicable.

the study for the calculation of the $BMDL_{10}$ because no other relevant studies on the carcinogenic properties of phenolphthalein were available. Whenever more precise toxicological data will become accessible, the calculated $BMDL_{10}$ value and resulting MOE values can be refined.

The MOE approach is recommended by EFSA and Joint FAO/WHO Expert Committee on Food Additives for priority setting by risk managers (EFSA 2005; Joint FAO/WHO Expert Committee on Food Additives 2005). EFSA (2005) considers that a MOE of 10 000 or higher, which is based on the $BMDL_{10}$ from an animal study, would be of low concern from a public health point of view and might be considered as a low priority for risk management actions. Use of four herbal supplements containing phenolphthalein (XIII, XVI, XVII and XIX) at the recommended daily doses would result in MOE values lower than 10 000 (96–220). However, assessing the risk from less-than-lifetime exposures and considering the use of the herbal supplements during a period of only a few weeks or months will result in MOE values which may be two to three orders of magnitude higher.

Side-effects of API levels found in the sampled herbal supplements

Results of the present study indicate that consumers of weight loss herbal supplements should be aware that these products can contain APIs. Because the current study was based on a targeted sampling protocol, the current results cannot be extrapolated to the entire market of weight loss food supplements. However, when the product produces significant results, such as weight loss, users should also be attentive to side-effects that might be caused by APIs. For sibutramine present in herbal supplements side-effects such as psychiatric symptoms are reported at dose levels in an estimated range of 2.8–60 mg (Taflinski & Chojnacka 2000; Litvan & Alcoverro-Fortuny 2007; Lee et al. 2008; Müller et al. 2009; Chen et al. 2010; Chong 2010; van Hunsel & van Grootheest 2011; Waszkiewicz et al. 2012). In our study sibutramine was found in 14 samples at levels that would result in estimated daily dose levels of 4–36 mg sibutramine day^{-1} when used according to recommendations. The lowest estimated daily dose level (4 mg day^{-1}) is above the lowest dose of this API at which side-effects are reported (2.8 mg), and based on this observation it is concluded that the use as recommended of these 14 supplements might induce side-effects. Furthermore, the use of six herbal supplements containing DMS (XXIII) or DDMS (VI–VIII, XIV and XV) as recommended might induce side-effects such as psychotic symptoms for DMS (Yuen et al. 2007; Chen et al. 2010) and cardiovascular effects for DDMS (Fil et al. 2011).

In conclusion, from the 50 herbal supplements sampled on the Dutch market, the use of 20 supplements as recommended would result in estimated daily dose

levels of sibutramine, DMS, DDMS and/or phenolphthalein that could produce pharmacological effects. Furthermore, use of four out of 10 herbal supplements as recommended would result in estimated daily phenolphthalein dose levels that would result in MOE values lower than 10 000. For short term-exposure (few weeks or months) MOE values may be two to three orders of magnitude higher. APIs should not be used as ingredients in food supplements. Such products should only be brought to the market as medicinal products because the legal framework for medicines is considerably better equipped to ensure the safe and effective use of these products. The current study shows that the use of food supplements with sibutramine, DMS, DDMS and/or phenolphthalein could result in pharmacological effects.

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