Ah receptor agonist activity in frequently consumed food items

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Food Types:	Many food types

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Table I. Estimated range of exposure of the Dutch population to NAhRAs from a number of frequently consumed food items, expressed as DR CALUX® pg TCDD-IEQ/day. The food items are prepared according to Dutch habits, and acidified to mimic the gastric environment. Levels based on the 24h incubations of the DR CALUX® bioassay.

VCP-2003 Product group	Samples	VCP-2003 Consumption g/day	Range pg TCDD-IEQ/g		Daily pg TCDD- IEQ/day	
			Lower	Upper	Lower	Upper
Potatoes	Mean of potatoes*	96	1.4	2.4	130	226
Leaf vegetables	Lettuce	15	0.0	0.1	0	2
Fruit vegetables	Bell pepper*	28	0.2	0.9	6	25
Tuberous vegetables	Carrot†	7	0.0	0.0	0	0
Crucifers	Mean of crucifers*	23	1.8	4.5	40	103
Peas, beans	Pea*	3	0.0	0.0	0	0
Flour	Wheat flour	1	0.8	0.8	1	1
Paste, rice	Mean of paste, rice*	46	0.7	0.7	31	32
Bread	Bread	151	0.4	14.5	60	2190
Fresh meat	Mean of fresh meat	44	0.8	1.1	36	47
Meat products	Hamburger‡	60	10.5	24.9	630	1494
Fish	Plaice (flatfish)‡	4	4.2	6.1	17	24
Fruit juices	Mean of fruit juices	159	3.7	13.1	594	2088
Total, pg/day					1545	6232

* Samples boiled

‡ Samples fried

Figure 1. Luciferase induction in rat H4IIEpGudluc1.1 cells determined by the DR CALUX[®] assay, expressed as % of the maximum induction by TCDD after 4, 8, 16 and 24 h of exposure to 3 times diluted extracts of a number of frequently consumed food items. Bars: mean of triplicate exposures $\pm$ sd.

Figure 2. DR CALUX[®] response in a dilution range of fried (black) and fried plus acidified (grey) French fries extracts. The exposure time was 24h. Bars: mean of triplicate exposures $\pm$ sd, * $P < 0.05$, ** $P < 0.01$

Figure 3. DR CALUX[®] response in extracts of raw (black), raw plus acidified (grey), boiled (white) and boiled plus acidified (striped) potatoes, bell pepper and cruciferous vegetables. The exposure time was 24h. Bars: mean of four (potatoes, undiluted), three (bell pepper, undiluted, three different color types), or eleven (cruciferous vegetables, three times diluted, four different types) samples of vegetables $\pm$ sd, * $P < 0.05$

Figure 4. DR CALUX[®] response in three times diluted extracts of boiled (black) and boiled plus acidified (grey) pastas, rice, corn and bread. The exposure time was 24h. Bars: mean of triplicate exposures $\pm$ sd, * $P < 0.05$

Figure 5. DR CALUX[®] response of undiluted extracts of raw (black), fried (grey) and fried plus acidified (striped) samples from minced beef, steak, pork, chicken breast, hamburger and flatfish (plaice). The exposure time was 24h. Bars: mean of triplicate exposures $\pm$ sd, * $P < 0.05$, ** $P < 0.01$

Figure 6. DR CALUX[®] response in a dilution range of orange juice (black), acidified orange juice (grey), grapefruit juice (white) and acidified grapefruit juice (striped) extracts. The exposure time was 24h. Bars: mean of seven (orange juice) of three (grapefruit juice) different types $\pm$ sd. At the 81 times dilution only grapefruit juice has been measured.

Figure 1.

Figure 2.

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Ah receptor agonist activity in frequently consumed food items

For Peer Review Only

Abstract

The aryl hydrocarbon receptor (AhR) receives much attention for its role in the toxicity of dioxins and dioxin-like PCBs. However, also many other compounds have been reported to bind and activate the AhR, of which natural food components are of special interest from a human health perspective. Using the DR CALUX[®] bioassay, we screened extracts from many food items, frequently consumed in The Netherlands, to estimate the intake of natural AhR-agonists (NAhRAs). Using the prototypical AhR-agonist TCDD as standard, we estimated that the daily intake of NAhRAs may be considerably higher than the reported intake of dioxins and dioxin-like PCBs. Potatoes, cruciferous vegetables, bread, hamburgers and grapefruit juice contained most NAhRAs. Food preparation and acid treatment can show a significant effect on the AhR-activation. Interaction of natural and xenobiotic AhR-agonists should be taken into account when performing risk-benefit analysis of both types of compounds.

Key words: *Ah receptor, TCDD, food, phytochemicals, DR CALUX[®]*

Introduction

The aryl hydrocarbon receptor (AhR) is a cytosolic ligand-activated transcription factor, which is present in many cell types of mammals, including humans. At the moment, there is still no consensus about the biological function of this receptor. However, it received much attention for its role in the toxicity of dioxins, especially the most potent activator 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Hankinson 1995; Safe 2001).

Not only contaminants like dioxins, PCBs and PAHs can bind to the AhR, but also a large number of compounds of natural origin can bind to and activate this receptor. Surprisingly, these compounds can have very different chemical structures, which indicates that this receptor has a rather promiscuous ligand binding site (Denison and Nagy 2003; Safe 1998). One of the most investigated natural AhR agonists (NAhRAs) is indole-3-carbinol (I3C), which is formed in cruciferous vegetables (Brussels sprouts, broccoli, cabbage) by an enzymatic reaction after crushing of the plant tissues. During consumption, I3C undergoes self-condensation into a number of oligomers and indolo[3,2-*b*]carbazole (ICZ) in the acid environment of the stomach. Although ICZ is formed in very low amounts (ca 0.0002%), its AhR activating potency is relatively high (Bjeldanes and others 1991). Also many other food items have been shown to contain relatively large amounts of NAhRAs, of which the chemical identity of the receptor-activating compounds is often unknown (Jeuken and others 2003).

In contrast with the prototypical AhR agonist TCDD and related toxic compounds, the NAhRAs do apparently not show clear toxic effects at normal dietary exposure levels; on the contrary, they even seem to have beneficial health effects. Many researchers attribute this to the induction of a number of phase I and II detoxifying biotransformation enzymes, like the cytochrome P450s CYP1A1, CYP1A2, GSTP, and

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4 NQO1 (Kim and Milner 2005; Rogan 2006). However, these enzymes are induced by
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6 the activation of the AhR, and are thus also induced by TCDD and other toxic dioxin-
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8 like contaminants. A distinctive property of the NAhRAs seems to be their rapid
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10 clearance from the body, resulting in an essentially transient effect on the expression of
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12 AhR-regulated genes as compared to the more persistent xenobiotic AhR agonists
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14 (Chen and others 1995; Pohjanvirta and others 2002). Another property that seems to
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16 distinguish many NAhRAs from e.g. dioxins is their ability to inhibit the enzymatic
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18 function of P450 enzymes, and even to inhibit AhR activation by TCDD (Amakura and
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20 others 2002; Pohl and others 2006). This raises the question whether humans are
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22 exposed to such an amount of NAhRAs that they could inhibit the possible toxic effects
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24 of compounds like dioxins and PAHs or that they may present a risk hazard because of
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26 their AhR agonist activity.
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33 The purpose of our study was to examine the AhR-activating properties of a number
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35 of food items frequently consumed in The Netherlands, in order to identify sources of
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37 NAhRAs and relate their intake by humans to that of dioxin-like AhR-activating food
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39 contaminants. We prepared the food samples in the way they are consumed normally by
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41 Dutch consumers, and we included treatment of the samples with hydrochloric acid
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43 (HCl) to mimic the acid environment of the stomach. A low pH could theoretically
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45 hydrolyse unstable NAhRAs and deactivate them, but on the other hand, some natural
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47 compounds, like I3C, can be transformed by acidification into relatively strong AhR
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49 agonists such as ICZ. Comparing the AhR-activation by the NAhRAs, as measured with
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51 the DR CALUX[®] assay, with the activity of TCDD, and relating this to the Dutch Food
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53 Consumption Survey of 2003 (Hulshof and others 2004), provided us with an estimation
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55 of the theoretical load of NAhRAs in the human diet. Because of the difficulty to
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measure every food item, this is likely to result in an under-estimation of the actual intake.

Materials and methods

Chemicals

Methanol, n-hexane, diethyl ether, sulphuric acid and silica gel 60 (70-230 mesh) were purchased from Merck (Darmstadt, Germany), luciferin assay mixture from Promega (Madison, WI, USA), cyclohexane and ethylacetate from Biosolve (Valkenswaard, The Netherlands).

Preparation of food extracts

All food items were bought at local supermarkets. Solid foods were grinded by a food chopper T71 (Moulinex, Woerden, The Netherlands). Five gram of fresh solid food or 10 ml of liquid food was mixed with 20 ml of methanol/water 85/15, and after 30 min of soaking, extracted twice with 20 ml of n-hexane/diethyl ether 97/3 by mixing for 1 h in an overhead mixer (REAX 2, Heidolph, Germany), followed by centrifugation at 3000 rpm for 10 min. Hexane-layers were collected, evaporated in a Speedvac (Hetavac VR-1, Birkerød, Denmark) and the residue dissolved in 40 µl of DMSO (Merck, Darmstadt, Germany). In order to mimic the acid environment of the stomach, in addition grinded samples were 1:1 mixed with 0.1 M HCl, adjusted to pH=1 with 1 M HCl (Merck, Darmstadt, Germany), and mixed for an hour before addition of the methanol/water. When relevant, samples were boiled before grinding according to common Dutch kitchen practises, and afterwards, also the boiling water was collected for examination. Extracts from food samples containing fat, like bread and meat, were

defatted in order to prevent incomplete mixing with the medium. Extract residues containing 40 μ l DMSO were dissolved in 15 ml of eluent (cyclohexane/ethylacetate 1/1 v/v), of which 12.5 ml were inserted into a gel permeation chromatography system (Gilson). Fat was separated by a BioBeads SX3 column (i.d. 2.5 cm, length 60 cm, 200-400 mesh), and the eluted fraction from 44 to 107 min was collected and evaporated until 40 μ l DMSO. To check for contamination with dioxin-like persistent AhR agonists, hexane-extracts of a number of samples were transferred on a column containing 10 g acid silica (33% H₂SO₄), on which NAhRAs are removed (Hoogenboom and others 2006b) and subsequently tested for residual AhR-agonist activity by DR CALUX[®] analysis.

Cell culture and treatment

DR CALUX[®] cells (rat H4IIE hepatoma cells stably transfected with pGudluc1.1, an AhR-regulated luciferase reporter plasmid) (Aarts and others 1995) were grown in α -MEM (Sigma, St-Louis, USA), containing 10% fetal bovine serum (GIBCO, Breda, The Netherlands) and 1% penicillin/streptomycin (Sigma), at 37°C and 5% CO₂. Cells were seeded 24 h before exposure in Costar 48 well-plates (5.10⁴ cells/well) and exposed for 24 h to sample solutions in DMSO added to the culture medium. During each experiment, a calibration curve was made of 0.5-500 pM TCDD (Schmidt BV, Amsterdam, The Netherlands). The undiluted sample concentration of the solid foods was equivalent to 1250 mg fresh product per ml exposure medium, except for the meat samples and fish, of which the concentration was 625 mg/ml. The undiluted concentration of the liquid samples was 2500 mg/ml. Samples were also diluted 3, 9, 27

or 81 times. The final concentration of DMSO in all exposures was 1.0 % (V/V). In addition, a number of samples were also exposed for 4, 8 and 16 h.

DR CALUX[®] assay

The Dioxin Receptor - Chemical-Activated LUCiferase gene eXpression assay was performed as described previously (Bovee and others 1998). After exposure of the cells in 48-well microtiter plates the medium was removed and cells were washed with PBS. Cells were lysed using Cell Culture Lysis Reagent (Promega), centrifuged and 20 µl of lysed solution of each well was transferred to a 96 wells plate. Luminescence was measured with a LuminoskanAscent (Thermo Labsystems, Finland) upon addition of 0.1 ml of luciferin assay mixture (20 mM Tricine, 1.07 mM (MgCO₃)₄Mg(OH)₂·5H₂O, 2.67 mM MgSO₄·7H₂O, 0.1 mM EDTA, 33.3 mM DTT, 261 µM Coenzyme A, 470 µM Luciferin, 530 µM ATP, pH 7.8). Using the program SlideWrite V6, the curve $y=a_0/(1+(x/a_1)^{a_2})$, which describes the AhR activation process suitably (Hoogenboom and others 2006a) was fitted for the data and the response maximum of TCDD and the TCDD luciferase induction equivalents (TCDD-IEQs) of the food extracts were calculated.

Statistics

Statistical analyses were performed by use of the 2-side Student's t-test. Effects of acidification or boiling of groups of foods were tested paired.

Results

By use of the DR CALUX[®] bioassay, the Ah-receptor activity was measured in extracts from a number of food items, regularly consumed in The Netherlands. To rule out the effects of possible contamination with dioxins and other persistent AhR agonists, a number of samples with relatively high luciferase induction has been examined by use of the acid silica clean up procedure which destroys the more labile natural AhR agonists. None of the acid silica treated samples showed a significant induction of the luciferase reporter gene expression, except for the flatfish, which showed a CALUX activity of about one-third of the non acid silica treated sample (results not shown). To establish whether the food extracts showed a similarly persistent response as TCDD, the CALUX cells were also exposed for 4, 8 and 16 h to a number of samples. Most of the samples induced luciferase 20-70 times more during 4 h and 8 h, than during 24 h of exposure, both at the 3 (Figure 1) and 27 times dilutions (results not shown). The 16 h exposures showed less difference with 24 h (1-3 times). Apparently, the AhR agonists in all samples are less persistent in activity than TCDD, probably because of their faster metabolism or faster increase in response compared with TCDD.

DR CALUX[®] response in food items

Figure 2 to 6 show the AhR activity in extracts of a number of food items frequently consumed in the Netherlands, as measured by the DR CALUX[®] assay after 24 h of exposure. All samples were tested in dilution ranges, but for clarity reasons not all dilutions are shown. The influence of acidification has been determined for most of the food samples, and where relevant according to the habitual Dutch food preparation, also the effect of boiling has been studied. The results are reviewed in the same order of product groups as described in the Dutch Food Consumption Survey of 2003 (Hulshof

and others 2004). The limit of detection of the used DR CALUX[®] assay is 0.5 pM TCDD (Hoogenboom and others 2006a), which corresponds with a luciferase induction of about 1% of the maximum induction caused by TCDD. In this study, a luciferase induction of 2% is assumed to be a significant activation of the AhR, corresponding to a limit of quantitation (LOQ) of 0.1 pg TCDD-luciferase induction equivalents (TCDD-IEQ) per gram of undiluted sample. After boiling the vegetables, the boiling water was collected and also examined for AhR activation, but the luciferase induction by these water extracts was negligible.

Potatoes. French fries contained a considerable amount of inducing compounds (up to 28% induction, Figure 2). At three dilutions, the acidification reduced the luciferase activity significantly by 20-30%. Potatoes showed a significant luciferase activity of about 4-7% of the TCDD maximum at the highest concentration. Boiled potatoes showed significant less induction than acidified raw potatoes (Figure 3).

Lettuce, carrot and pea. The DR CALUX[®] response of these vegetables was near or below 1% of TCDD maximum at the highest concentration (results not shown).

Bell pepper. Three different color types of bell pepper were examined. Undiluted samples showed an activity of 4-6 % of TCDD maximum. Acidification of the boiled vegetables increased the activity significantly (Figure 3).

Cruciferous vegetables. Four types of cruciferous vegetables have been examined: Brussels sprouts, broccoli, Savoy cabbage and white cabbage. All of them showed a relatively strong luciferase induction. Although Brussels sprouts tended to be the most strongly activating vegetable, the differences were not significant and the mean of all types has been plotted in Figure 3. As expected in view of the known acid induced NAhRAs like I3C in cruciferous vegetables, acidification increased the response

significantly by 2.8 (acidified vs raw) to 2 (boiled and acidified vs raw) times. Boiling did not have a large effect on luciferase induction.

Paste, rice and corn. Penne, macaroni, spaghetti and rice but not corn could induce the DR CALUX[®] response by 2-7%. Acidification increased the response of penne, and decreased the response of rice significantly (Figure 4).

Bread. Bread showed a significant response of 15-20% at the highest concentration (Figure 4). Acidification decreased the response, but not significantly. Wheat flour was very low in AhR activity (results not shown).

Meat and fish. Very high levels of AhR activity, up to 80% of the TCDD maximum, were observed with hamburgers and the flatfish plaice, and to a lesser extent with fried minced beef (Figure 5). Raw minced beef showed no significant response and frying also increased the response of chicken breast and hamburger, but decreased the response of steak significantly. Acidification of the fried samples had no significant influence on the luciferase induction.

Fruit juices. Undiluted orange juice showed a moderate activity of up to 30% of the TCDD maximum, while grapefruit juice highly induced the luciferase activity up to ca. 40% at low concentrations (Figure 6). Undiluted samples of grapefruit juice extracts were clearly cytotoxic, and also the 3-times dilutions were less active. Acidification of these fruit juices had no significant effect on the AhR induction, probably also because of the low pH of the juices themselves (pH=3.2).

Human exposure with NAhRAs

To estimate the amount of AhR-activating substances found in the human diet, we calculated their contribution to the DR CALUX[®] induction relative to the induction by

TCDD, and expressed it as pg TCDD luciferase induction-equivalents (TCDD-IEQ, Table I). In this way TCDD has been used as a normalization standard for AhR-activity as measured by DR CALUX[®], enabling comparison with AhR-agonist levels in food items reported in literature. We emphasize that this does not imply a comparison to TCDD-toxicity. It was decided to use only the 24 h exposure data. Because the slope of the dose-response curves varies considerably between food extracts and TCDD, lower and upper limits of the DR CALUX[®] inductions were determined over the range of food extract dilutions tested, and the means of the representatives of the product groups are compared with the mean daily consumptions of the food items as reported in the Dutch Food Consumption Survey of 2003 (Hulshof and others 2004). Where appropriate, only acidified and boiled samples were taken into account. Table I shows that the total TCDD-IEQ amounts to 1545 pg/day at the lowest estimation, and 6235 pg/day at the highest. Of all tested food extracts, potatoes, cruciferous vegetables, bread, hamburgers and fruit juices are the most important sources of AhR agonist activity for the average consumer. The 81-times diluted grapefruit juice extract showed the highest relative DR CALUX[®] activity of 41 pg TCDD-IEQ per g of product (results not shown). Because of the relatively small sampling of food items in our study, the overall exposure of the Dutch people to these kind of bioactive compounds will probably be significantly higher.

Discussion

Many compounds of natural origin appear to be AhR-agonists and extracts of many food items were found to have a significant influence on the AhR activity as measured

by *in vitro* and *in vivo* assays (Amakura and others 2002; Amakura and others 2003; Jeuken and others 2003; Leibelt and others 2003; Nishiumi and others 2006; Pohl and others 2006). Some of these compounds and mixtures showed both activating and inhibiting effects on the AhR pathway. Very high estimates of AhR activating potential by natural food components have been reported (Jeuken and others 2003; Safe 1998). However, a systematic inventory of the exposure of humans to NAhRAs in food has not been made. By analyzing a variety of food items frequently taken by Dutch consumers, and including the effect of food preparation and acidification, we showed in this study that the intake of NAhRAs through the diet is probably not as high as previously thought (Jeuken and others 2003; Safe 1998), but still much higher than the intake of persistent xenobiotic AhR agonists (Baars and others 2004).

French fries, cruciferous vegetables, bread, hamburgers and citrus juices showed the most important AhR agonist activity of the food extracts we examined. Many natural compounds which can cause the AhR-activity in these food items may not have been identified yet. In cruciferous vegetables, the AhR agonists are attributed to the acid reaction products of indole-3-carbinol (I3C). In baked or fried foods, like fries, bread and meat products, the activity may be caused by compounds which originate from the formation of polycyclic aromatic hydrocarbons (PAHs), heterocyclic amines or Maillard products during the food preparation process at high temperatures. Also blood rests in meat may contribute to the AhR activity; heme degradation products like bilirubin and biliverdin are reported as AhR agonists. Polyphenols and furocoumarins are probably responsible for the shown activity in citrus fruit products (Denison and Nagy 2003).

Expressing the levels of NAhRAs in food in TCDD-IEQs may suggest that the responsible components represent a certain level of AhR-mediated toxicity according to

the TEQ (toxic or dioxin equivalent) approach as described by Safe (Safe 1998). However, we emphasize that we only used TCDD-IEQs to compare AhR activity, because the NAhRAs do probably not meet some of the important requirements for the TEQ-principle, namely additivity, and more important, persistence (Safe 1998). However, TCDD is a very potent agonist, and shows very reproducible results in the DR-CALUX[®] assay, which makes it an ideal standard for the expression of relative AhR agonist activity. This implies that our results can not be interpreted as direct measurements of toxicity of food components, but only reveal the AhR activation effects, expressed as TCDD-IEQs or luciferase induction equivalents. Because the slopes of the dose-response curve of NAhRAs and PAHs differ from that of dioxins, the dilution of the samples has an important effect on the results of the calculation of pg TCDD-IEQs/g sample (Windal and others 2005). Because of the probably fragile nature of many natural compounds, it is important to include effects like food preparation and the effect of acidification in the gastric environment on the levels of AhR agonist activity. Frying and acidification can have a significant influence on AhR activity, boiling to a much lesser extent and the loss of AhR agonists in the boiling water was negligible.

The exposure time is another important factor in AhR activity measurement. Natural compounds, but also some PAHs like benzo[a]pyrene, can be metabolized very quickly in comparison with TCDD (Chen and others 1995; Machala and others 2001; Pohjanvirta and others 2002). In our study, the luciferase induction after 4 h exposure to TCDD has reached only about 30% of its maximum after 24 h, whereas the induction by PAHs and NAhRAs is higher at 4h than at 24h of exposure. As a result, a relatively short exposure time of 4 h will show a much higher luciferase induction by NAhRAs

and PAHs compared with TCDD, than after 24 h of exposure. This may explain the large difference between some results of Jeuken *et al.* (2003) and ours. They found e.g. 640 pg TCDD-IEQ/g in potato, where we found 1.4-2.4 pg TCDD-IEQ/g (Table I). However, based on our measurement with the 4h exposure time, we found in line with Jeuken *et al.* a much higher amount of 110-332 pg TCDD-IEQ/g in potato. The interpretation of DR-CALUX[®] measurements on extracts of food items is thus very complicated, and we do not pretend to give a conclusive opinion about their eventual toxic potential in the human diet. Important, however, is to obtain an estimation which shows whether the exposure to NAhRAs is considerably higher than to xenobiotic AhR agonists such as dioxins and other persistent compounds in the human diet, or much lower and negligible. In 1999, the estimated intake of the sum of dioxins and dioxin-like PCBs in The Netherlands was 1.2 pg WHO-TEQ per kg body weight (bw) per day, which would be for a person weighing 70 kg 84 pg WHO-TEQ. Of this population was 8% exposed to intake levels above the tolerable weekly intake for dioxins and dioxin-like PCBs of 14 pg WHO-TEQ per kg bw per week, or over 140 pg WHO-TEQ per person per day. The average dietary intake of these xenobiotic AhR agonists in 1978 was nearly 700 pg WHO-TEQ per person per day (Baars and others 2004). These levels are that far below the theoretical levels estimated by Jeuken *et al.* (e.g. 96000 pg TCDD-IEQ per serving of 150 g potato), or our study (1545-6235 pg TCDD-IEQ/day for part of the average daily diet), that the regular intake of NAhRAs should be seriously evaluated with regard to the biological meaning of the level of AhR activation that they may cause. On the one hand, the omnipresence of the competing natural AhR agonists, together with the presence of many AhR antagonists (Amakura and others 2002; Amakura and others 2003; Baumgart and others 2005) in food, may appear as a

prophylaxis against xenobiotic intoxication via the AhR. On the other hand, several human studies showed AhR responsive effects, like CYP1A2 induction, by several non-dioxin like AhR agonists such as NAhRAs from cruciferous vegetables (Kall and others 1996; Murray and others 2001), I3C (Reed and others 2005), the anti-ulcer drug omeprazole (Rost and others 1992), and heterocyclic aromatic amines (Sinha and others 1994). This seems to be in contrast with a regular exposure to numerous AhR agonists in the human diet, which would constantly elevate the AhR activity. Another major problem in this is, that the exact pathways by which the xenobiotic dioxins and dioxin-like PCBs exert their toxicity are not well understood (Guzelian and others 2006; Steenland and others 2004). This makes it very difficult to understand to what extent NAhRAs, in amounts effective to cause AhR related effects, e.g. CYP1A2 induction by concentrated food additives, also can initiate dioxin-like toxic pathways, especially when slow processes like tumor promotion are involved. Besides tumor suppressing capacity, high doses of NAhRAs given to laboratory animals have sometimes also shown tumor promoting capacity (Dashwood 1998), and the accumulation effect of persistent fat-soluble xenobiotic AhR agonists could be mimicked by constant or regular NAhRA exposure.

In conclusion, this study confirms to a certain extent that the human diet contains a large amount of AhR-activating substances of natural origin, which triggers questions about the potential effects in humans, also in relation to the intake and adverse effects of xenobiotic dioxins and dioxin-like PCBs. In order to elucidate the potential toxicity or health-beneficial properties of these relatively large amounts of bioactive dietary components, the toxic pathway(s) of dioxins in humans should be elucidated first. This would give indications at what critical steps of the toxic pathway(s) the NAhRAs would

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4 have a significant dioxin-like toxic effect or on the contrary, induce totally different or
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6 even beneficial health effects, and what ultimately the predominant effect or the balance
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8 of all these effects would be.
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11 12 13 14 15 16 **Acknowledgement** 17

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