



HAL
open science

Ah receptor agonist activity in frequently consumed food items

Wilhelm Johan de Waard, Jac M.M.J.G. Aarts, Ad A.C.M. Peijnenburg, Theo M.C.M. de Kok, Frederik-Jan van Schooten, Ron Laurentius Hoogenboom

► **To cite this version:**

Wilhelm Johan de Waard, Jac M.M.J.G. Aarts, Ad A.C.M. Peijnenburg, Theo M.C.M. de Kok, Frederik-Jan van Schooten, et al.. Ah receptor agonist activity in frequently consumed food items. Food Additives and Contaminants, 2008, 25 (06), pp.779-787. 10.1080/02652030701798880. hal-00577440

HAL Id: hal-00577440

<https://hal.science/hal-00577440>

Submitted on 17 Mar 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Ah receptor agonist activity in frequently consumed food items

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2007-328.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	08-Nov-2007
Complete List of Authors:	de Waard, Wilhelm; RIVM, Safety Medicines and Teratology Aarts, Jac; Wageningen University, Toxicology Peijnenburg, Ad; RIKILT-Institute of Food Safety de Kok, Theo; Maastricht University van Schooten, Frederik-Jan; Maastricht University Hoogenboom, Ron; RIKILT-Institute of Food Safety, Safety and Health
Methods/Techniques:	Screening - Calux
Additives/Contaminants:	Dioxins - TEQs
Food Types:	Many food types

SCHOLARONE™
Manuscripts

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.

Deleted: standards

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

Formatted: Superscript

Formatted: Superscript

Formatted: Font: 10 pt, Superscript

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Superscript

Formatted: Font: 10 pt

* Samples boiled

‡ Samples fried

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

1
2
3
4 Figure 6. DR CALUX[®] response in a dilution range of orange juice (black), acidified
5 orange juice (grey), grapefruit juice (white) and acidified grapefruit juice (striped)
6
7 orange juice (grey), grapefruit juice (white) and acidified grapefruit juice (striped)
8
9 extracts. The exposure time was 24h. Bars: mean of seven (orange juice) of three
10
11 (grapefruit juice) different types \pm sd. At the 81 times dilution only grapefruit juice has
12
13 been measured.
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review Only

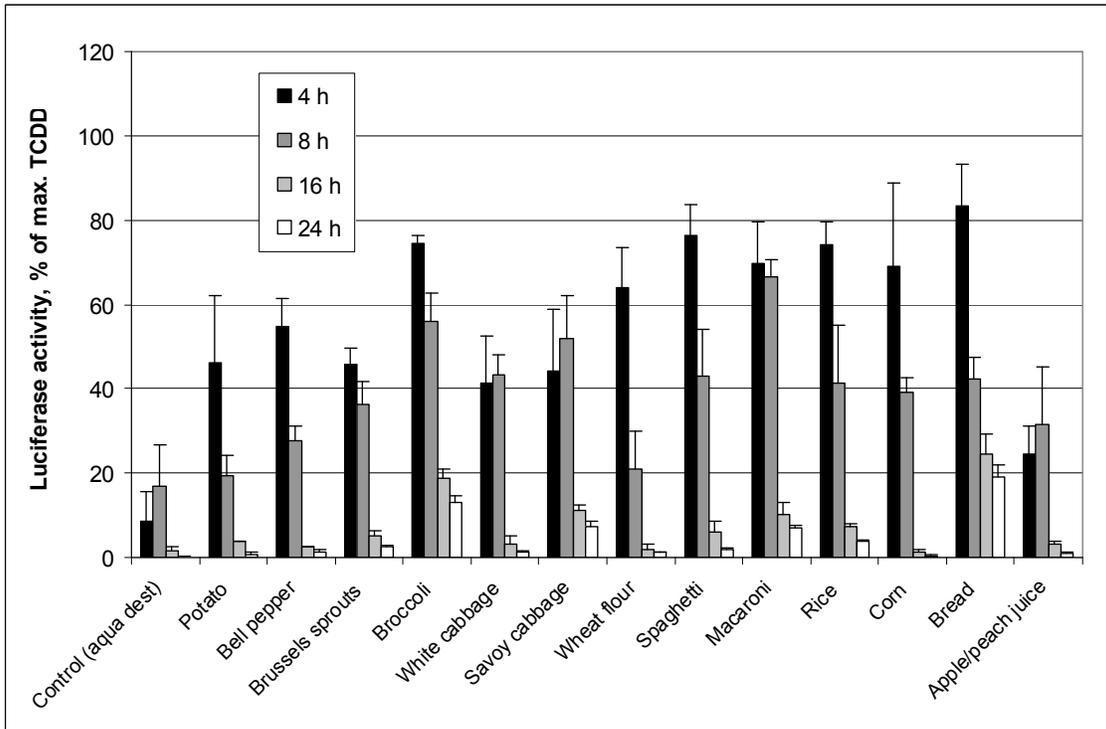


Figure 1.

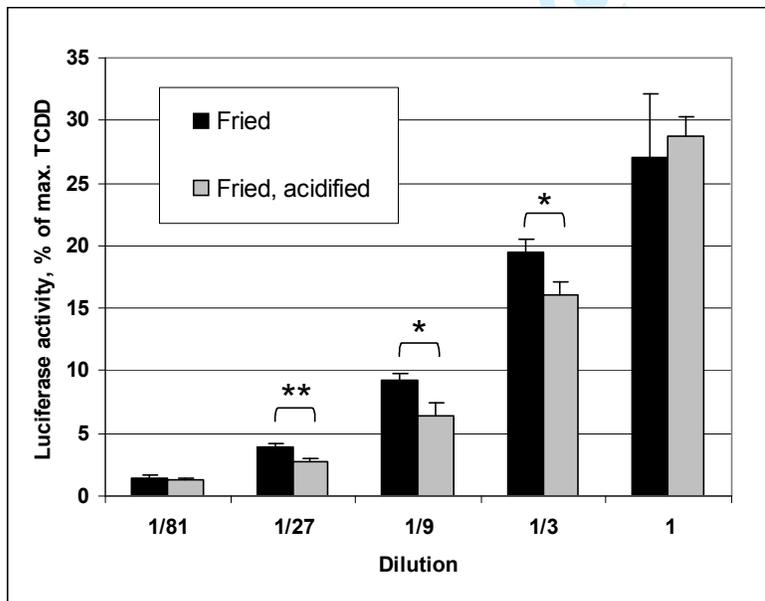


Figure 2.

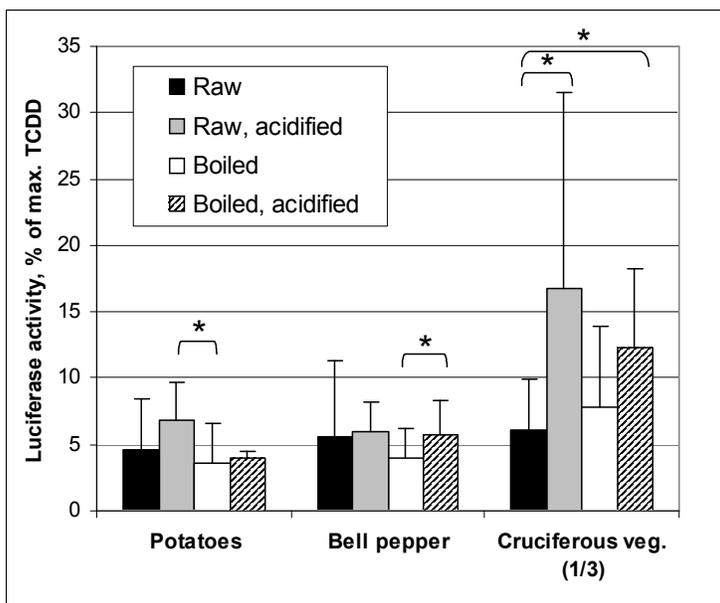


Figure 3.

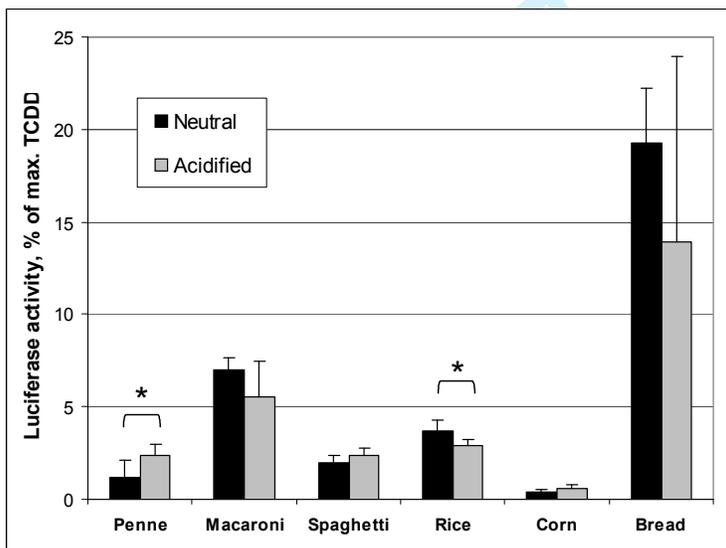


Figure 4.

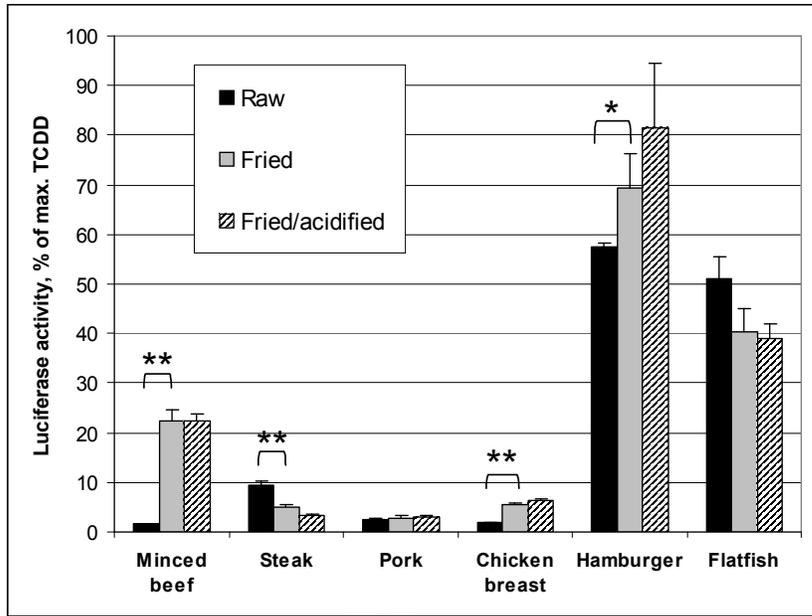


Figure 5.

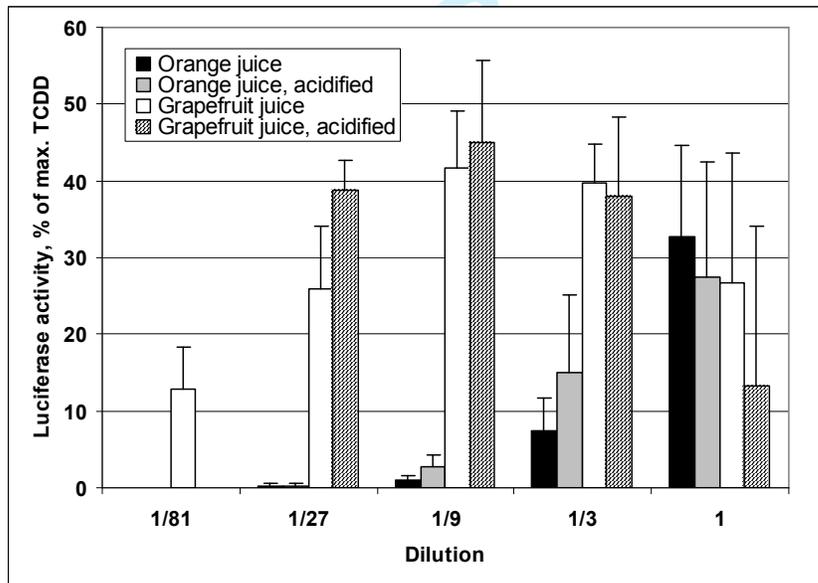


Figure 6.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

1
2
3
4 NQO1 (Kim and Milner 2005; Rogan 2006). However, these enzymes are induced by
5
6 the activation of the AhR, and are thus also induced by TCDD and other toxic dioxin-
7
8 like contaminants. A distinctive property of the NAhRAs seems to be their rapid
9
10 clearance from the body, resulting in an essentially transient effect on the expression of
11
12 AhR-regulated genes as compared to the more persistent xenobiotic AhR agonists
13
14 (Chen and others 1995; Pohjanvirta and others 2002). Another property that seems to
15
16 distinguish many NAhRAs from e.g. dioxins is their ability to inhibit the enzymatic
17
18 function of P450 enzymes, and even to inhibit AhR activation by TCDD (Amakura and
19
20 others 2002; Pohl and others 2006). This raises the question whether humans are
21
22 exposed to such an amount of NAhRAs that they could inhibit the possible toxic effects
23
24 of compounds like dioxins and PAHs or that they may present a risk hazard because of
25
26 their AhR agonist activity.
27
28
29
30
31

32
33 The purpose of our study was to examine the AhR-activating properties of a number
34
35 of food items frequently consumed in The Netherlands, in order to identify sources of
36
37 NAhRAs and relate their intake by humans to that of dioxin-like AhR-activating food
38
39 contaminants. We prepared the food samples in the way they are consumed normally by
40
41 Dutch consumers, and we included treatment of the samples with hydrochloric acid
42
43 (HCl) to mimic the acid environment of the stomach. A low pH could theoretically
44
45 hydrolyse unstable NAhRAs and deactivate them, but on the other hand, some natural
46
47 compounds, like I3C, can be transformed by acidification into relatively strong AhR
48
49 agonists such as ICZ. Comparing the AhR-activation by the NAhRAs, as measured with
50
51 the DR CALUX[®] assay, with the activity of TCDD, and relating this to the Dutch Food
52
53 Consumption Survey of 2003 (Hulshof and others 2004), provided us with an estimation
54
55 of the theoretical load of NAhRAs in the human diet. Because of the difficulty to
56
57
58
59
60

1
2
3
4 measure every food item, this is likely to result in an under-estimation of the actual
5
6 intake.
7
8
9

10 11 **Materials and methods**

12 13 *Chemicals*

14
15 Methanol, n-hexane, diethyl ether, sulphuric acid and silica gel 60 (70-230 mesh) were
16
17 purchased from Merck (Darmstadt, Germany), luciferin assay mixture from Promega
18
19 (Madison, WI, USA), cyclohexane and ethylacetate from Biosolve (Valkenswaard, The
20
21 Netherlands).
22
23
24
25

26 27 28 *Preparation of food extracts*

29
30 All food items were bought at local supermarkets. Solid foods were grinded by a food
31
32 chopper T71 (Moulinex, Woerden, The Netherlands). Five gram of fresh solid food or
33
34 10 ml of liquid food was mixed with 20 ml of methanol/water 85/15, and after 30 min
35
36 of soaking, extracted twice with 20 ml of n-hexane/diethyl ether 97/3 by mixing for 1 h
37
38 in an overhead mixer (REAX 2, Heidolph, Germany), followed by centrifugation at
39
40 3000 rpm for 10 min. Hexane-layers were collected, evaporated in a Speedvac (Hetavac
41
42 VR-1, Birkerod, Denmark) and the residue dissolved in 40 μ l of DMSO (Merck,
43
44 Darmstadt, Germany). In order to mimic the acid environment of the stomach, in
45
46 addition grinded samples were 1:1 mixed with 0.1 M HCl, adjusted to pH=1 with 1 M
47
48 HCl (Merck, Darmstadt, Germany), and mixed for an hour before addition of the
49
50 methanol/water. When relevant, samples were boiled before grinding according to
51
52 common Dutch kitchen practises, and afterwards, also the boiling water was collected
53
54 for examination. Extracts from food samples containing fat, like bread and meat, were
55
56
57
58
59
60

1
2
3
4 defatted in order to prevent incomplete mixing with the medium. Extract residues
5
6 containing 40 µl DMSO were dissolved in 15 ml of eluent (cyclohexane/ethylacetate 1/1
7
8 v/v), of which 12.5 ml were inserted into a gel permeation chromatography system
9
10 (Gilson). Fat was separated by a BioBeads SX3 column (i.d. 2.5 cm, length 60 cm, 200-
11
12 400 mesh), and the eluted fraction from 44 to 107 min was collected and evaporated
13
14 until 40 µl DMSO. To check for contamination with dioxin-like persistent AhR
15
16 agonists, hexane-extracts of a number of samples were transferred on a column
17
18 containing 10 g acid silica (33% H₂SO₄), on which NAhRAs are removed
19
20 (Hoogenboom and others 2006b) and subsequently tested for residual AhR-agonist
21
22 activity by DR CALUX[®] analysis.
23
24
25
26
27
28
29

30 *Cell culture and treatment*

31
32 DR CALUX[®] cells (rat H4IIE hepatoma cells stably transfected with pGudluc1.1, an
33
34 AhR-regulated luciferase reporter plasmid) (Aarts and others 1995) were grown in α-
35
36 MEM (Sigma, St-Louis, USA), containing 10% fetal bovine serum (GIBCO, Breda,
37
38 The Netherlands) and 1% penicillin/streptomycin (Sigma), at 37°C and 5% CO₂. Cells
39
40 were seeded 24 h before exposure in Costar 48 well-plates (5.10⁴ cells/well) and
41
42 exposed for 24 h to sample solutions in DMSO added to the culture medium. During
43
44 each experiment, a calibration curve was made of 0.5-500 pM TCDD (Schmidt BV,
45
46 Amsterdam, The Netherlands). The undiluted sample concentration of the solid foods
47
48 was equivalent to 1250 mg fresh product per ml exposure medium, except for the meat
49
50 samples and fish, of which the concentration was 625 mg/ml. The undiluted
51
52 concentration of the liquid samples was 2500 mg/ml. Samples were also diluted 3, 9, 27
53
54
55
56
57
58
59
60

1
2
3
4 or 81 times. The final concentration of DMSO in all exposures was 1.0 % (V/V). In
5
6 addition, a number of samples were also exposed for 4, 8 and 16 h.
7
8
9

10 11 *DR CALUX[®] assay*

12
13 The Dioxin Receptor - Chemical-Activated LUCiferase gene eXpression assay was
14
15 performed as described previously (Bovee and others 1998). After exposure of the cells
16
17 in 48-well microtiter plates the medium was removed and cells were washed with PBS.
18
19 Cells were lysed using Cell Culture Lysis Reagent (Promega), centrifuged and 20 µl of
20
21 lysed solution of each well was transferred to a 96 wells plate. Luminescence was
22
23 measured with a LuminoskanAscent (Thermo LabSystems, Finland) upon addition of
24
25 0.1 ml of luciferin assay mixture (20 mM Tricine, 1.07 mM (MgCO₃)₄Mg(OH)₂·5H₂O,
26
27 2.67 mM MgSO₄·7H₂O, 0.1 mM EDTA, 33.3 mM DTT, 261 µM Coenzyme A, 470
28
29 µM Luciferin, 530 µM ATP, pH 7.8). Using the program SlideWrite V6, the curve
30
31 $y=a_0/(1+(x/a_1)^{a_2})$, which describes the AhR activation process suitably (Hoogenboom
32
33 and others 2006a) was fitted for the data and the response maximum of TCDD and the
34
35 TCDD luciferase induction equivalents (TCDD-IEQs) of the food extracts were
36
37 calculated.
38
39
40
41
42
43
44
45
46

47 *Statistics*

48
49 Statistical analyses were performed by use of the 2-side Student's t-test. Effects of
50
51 acidification or boiling of groups of foods were tested paired.
52
53
54
55

56 **Results**

1
2
3
4 By use of the DR CALUX[®] bioassay, the Ah-receptor activity was measured in extracts
5
6 from a number of food items, regularly consumed in The Netherlands. To rule out the
7
8 effects of possible contamination with dioxins and other persistent AhR agonists, a
9
10 number of samples with relatively high luciferase induction has been examined by use
11
12 of the acid silica clean up procedure which destroys the more labile natural AhR
13
14 agonists. None of the acid silica treated samples showed a significant induction of the
15
16 luciferase reporter gene expression, except for the flatfish, which showed a CALUX
17
18 activity of about one-third of the non acid silica treated sample (results not shown). To
19
20 establish whether the food extracts showed a similarly persistent response as TCDD, the
21
22 CALUX cells were also exposed for 4, 8 and 16 h to a number of samples. Most of the
23
24 samples induced luciferase 20-70 times more during 4 h and 8 h, than during 24 h of
25
26 exposure, both at the 3 (Figure 1) and 27 times dilutions (results not shown). The 16 h
27
28 exposures showed less difference with 24 h (1-3 times). Apparently, the AhR agonists
29
30 in all samples are less persistent in activity than TCDD, probably because of their faster
31
32 metabolism or faster increase in response compared with TCDD.
33
34
35
36
37
38
39
40
41

42 *DR CALUX[®] response in food items*

43
44 Figure 2 to 6 show the AhR activity in extracts of a number of food items frequently
45
46 consumed in the Netherlands, as measured by the DR CALUX[®] assay after 24 h of
47
48 exposure. All samples were tested in dilution ranges, but for clarity reasons not all
49
50 dilutions are shown. The influence of acidification has been determined for most of the
51
52 food samples, and where relevant according to the habitual Dutch food preparation, also
53
54 the effect of boiling has been studied. The results are reviewed in the same order of
55
56 product groups as described in the Dutch Food Consumption Survey of 2003 (Hulshof
57
58
59
60

1
2
3
4 and others 2004). The limit of detection of the used DR CALUX[®] assay is 0.5 pM
5
6 TCDD (Hoogenboom and others 2006a), which corresponds with a luciferase induction
7
8 of about 1% of the maximum induction caused by TCDD. In this study, a luciferase
9
10 induction of 2% is assumed to be a significant activation of the AhR, corresponding to a
11
12 limit of quantitation (LOQ) of 0.1 pg TCDD-luciferase induction equivalents (TCDD-
13
14 IEQ) per gram of undiluted sample. After boiling the vegetables, the boiling water was
15
16 collected and also examined for AhR activation, but the luciferase induction by these
17
18 water extracts was negligible.
19
20
21
22

23 *Potatoes.* French fries contained a considerable amount of inducing compounds (up
24
25 to 28% induction, Figure 2). At three dilutions, the acidification reduced the luciferase
26
27 activity significantly by 20-30%. Potatoes showed a significant luciferase activity of
28
29 about 4-7% of the TCDD maximum at the highest concentration. Boiled potatoes
30
31 showed significant less induction than acidified raw potatoes (Figure 3).
32
33
34

35 *Lettuce, carrot and pea.* The DR CALUX[®] response of these vegetables was near or
36
37 below 1% of TCDD maximum at the highest concentration (results not shown).
38
39

40 *Bell pepper.* Three different color types of bell pepper were examined. Undiluted
41
42 samples showed an activity of 4-6 % of TCDD maximum. Acidification of the boiled
43
44 vegetables increased the activity significantly (Figure 3).
45
46

47 *Cruciferous vegetables.* Four types of cruciferous vegetables have been examined:
48
49 Brussels sprouts, broccoli, Savoy cabbage and white cabbage. All of them showed a
50
51 relatively strong luciferase induction. Although Brussels sprouts tended to be the most
52
53 strongly activating vegetable, the differences were not significant and the mean of all
54
55 types has been plotted in Figure 3. As expected in view of the known acid induced
56
57 NAhRAs like I3C in cruciferous vegetables, acidification increased the response
58
59
60

1
2
3
4 significantly by 2.8 (acidified vs raw) to 2 (boiled and acidified vs raw) times. Boiling did
5
6 not have a large effect on luciferase induction.
7

8
9 *Paste, rice and corn.* Penne, macaroni, spaghetti and rice but not corn could induce
10 the DR CALUX[®] response by 2-7%. Acidification increased the response of penne, and
11 decreased the response of rice significantly (Figure 4).
12
13

14
15 *Bread.* Bread showed a significant response of 15-20% at the highest concentration
16 (Figure 4). Acidification decreased the response, but not significantly. Wheat flour was
17 very low in AhR activity (results not shown).
18
19

20
21 *Meat and fish.* Very high levels of AhR activity, up to 80% of the TCDD maximum,
22 were observed with hamburgers and the flatfish plaice, and to a lesser extent with fried
23 minced beef (Figure 5). Raw minced beef showed no significant response and frying
24 also increased the response of chicken breast and hamburger, but decreased the response
25 of steak significantly. Acidification of the fried samples had no significant influence on
26 the luciferase induction.
27
28

29
30 *Fruit juices.* Undiluted orange juice showed a moderate activity of up to 30% of the
31 TCDD maximum, while grapefruit juice highly induced the luciferase activity up to ca.
32 40% at low concentrations (Figure 6). Undiluted samples of grapefruit juice extracts
33 were clearly cytotoxic, and also the 3-times dilutions were less active. Acidification of
34 these fruit juices had no significant effect on the AhR induction, probably also because
35 of the low pH of the juices themselves (pH=3.2).
36
37

38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 *Human exposure with NAhRAs*

55
56 To estimate the amount of AhR-activating substances found in the human diet, we
57
58 calculated their contribution to the DR CALUX[®] induction relative to the induction by
59
60

1
2
3
4 TCDD, and expressed it as pg TCDD luciferase induction-equivalents (TCDD-IEQ,
5
6 Table I). In this way TCDD has been used as a normalization standard for AhR-activity
7
8 as measured by DR CALUX[®], enabling comparison with AhR-agonist levels in food
9
10 items reported in literature. We emphasize that this does not imply a comparison to
11
12 TCDD-toxicity. It was decided to use only the 24 h exposure data. Because the slope of
13
14 the dose-response curves varies considerably between food extracts and TCDD, lower
15
16 and upper limits of the DR CALUX[®] inductions were determined over the range of food
17
18 extract dilutions tested, and the means of the representatives of the product groups are
19
20 compared with the mean daily consumptions of the food items as reported in the Dutch
21
22 Food Consumption Survey of 2003 (Hulshof and others 2004). Where appropriate, only
23
24 acidified and boiled samples were taken into account. Table I shows that the total
25
26 TCDD-IEQ amounts to 1545 pg/day at the lowest estimation, and 6235 pg/day at the
27
28 highest. Of all tested food extracts, potatoes, cruciferous vegetables, bread, hamburgers
29
30 and fruit juices are the most important sources of AhR agonist activity for the average
31
32 consumer. The 81-times diluted grapefruit juice extract showed the highest relative DR
33
34 CALUX[®] activity of 41 pg TCDD-IEQ per g of product (results not shown). Because of
35
36 the relatively small sampling of food items in our study, the overall exposure of the
37
38 Dutch people to these kind of bioactive compounds will probably be significantly
39
40 higher.
41
42
43
44
45
46
47
48
49
50
51
52
53

54 Discussion

55
56 Many compounds of natural origin appear to be AhR-agonists and extracts of many
57
58 food items were found to have a significant influence on the AhR activity as measured
59
60

1
2
3
4 by *in vitro* and *in vivo* assays (Amakura and others 2002; Amakura and others 2003;
5
6 Jeuken and others 2003; Leibel and others 2003; Nishiumi and others 2006; Pohl and
7
8 others 2006). Some of these compounds and mixtures showed both activating and
9
10 inhibiting effects on the AhR pathway. Very high estimates of AhR activating potential
11
12 by natural food components have been reported (Jeuken and others 2003; Safe 1998).
13
14 However, a systematic inventory of the exposure of humans to NAhRAs in food has not
15
16 been made. By analyzing a variety of food items frequently taken by Dutch consumers,
17
18 and including the effect of food preparation and acidification, we showed in this study
19
20 that the intake of NAhRAs through the diet is probably not as high as previously
21
22 thought (Jeuken and others 2003; Safe 1998), but still much higher than the intake of
23
24 persistent xenobiotic AhR agonists (Baars and others 2004).
25
26
27
28
29

30 French fries, cruciferous vegetables, bread, hamburgers and citrus juices showed the
31
32 most important AhR agonist activity of the food extracts we examined. Many natural
33
34 compounds which can cause the AhR-activity in these food items may not have been
35
36 identified yet. In cruciferous vegetables, the AhR agonists are attributed to the acid
37
38 reaction products of indole-3-carbinol (I3C). In baked or fried foods, like fries, bread
39
40 and meat products, the activity may be caused by compounds which originate from the
41
42 formation of polycyclic aromatic hydrocarbons (PAHs), heterocyclic amines or Maillard
43
44 products during the food preparation process at high temperatures. Also blood rests in
45
46 meat may contribute to the AhR activity; heme degradation products like bilirubin and
47
48 biliverdin are reported as AhR agonists. Polyphenols and furocoumarins are probably
49
50 responsible for the shown activity in citrus fruit products (Denison and Nagy 2003).
51
52
53
54
55

56 Expressing the levels of NAhRAs in food in TCDD-IEQs may suggest that the
57
58 responsible components represent a certain level of AhR-mediated toxicity according to
59
60

1
2
3
4 the TEQ (toxic or dioxin equivalent) approach as described by Safe (Safe 1998).
5
6
7 However, we emphasize that we only used TCDD-IEQs to compare AhR activity,
8
9 because the NAhRAs do probably not meet some of the important requirements for the
10
11 TEQ-principle, namely additivity, and more important, persistence (Safe 1998).
12
13 However, TCDD is a very potent agonist, and shows very reproducible results in the
14
15 DR-CALUX[®] assay, which makes it an ideal standard for the expression of relative
16
17 AhR agonist activity. This implies that our results can not be interpreted as direct
18
19 measurements of toxicity of food components, but only reveal the AhR activation
20
21 effects, expressed as TCDD-IEQs or luciferase induction equivalents. Because the
22
23 slopes of the dose-response curve of NAhRAs and PAHs differ from that of dioxins, the
24
25 dilution of the samples has an important effect on the results of the calculation of pg
26
27 TCDD-IEQs/g sample (Windal and others 2005). Because of the probably fragile
28
29 nature of many natural compounds, it is important to include effects like food
30
31 preparation and the effect of acidification in the gastric environment on the levels of
32
33 AhR agonist activity. Frying and acidification can have a significant influence on AhR
34
35 activity, boiling to a much lesser extent and the loss of AhR agonists in the boiling
36
37 water was negligible.
38
39
40
41
42
43

44 The exposure time is another important factor in AhR activity measurement. Natural
45
46 compounds, but also some PAHs like benzo[*a*]pyrene, can be metabolized very quickly
47
48 in comparison with TCDD (Chen and others 1995; Machala and others 2001;
49
50 Pohjanvirta and others 2002). In our study, the luciferase induction after 4 h exposure to
51
52 TCDD has reached only about 30% of its maximum after 24 h, whereas the induction
53
54 by PAHs and NAhRAs is higher at 4h than at 24h of exposure. As a result, a relatively
55
56 short exposure time of 4 h will show a much higher luciferase induction by NAhRAs
57
58
59
60

1
2
3
4 and PAHs compared with TCDD, than after 24 h of exposure. This may explain the
5
6 large difference between some results of Jeuken *et al.* (2003) and ours. They found e.g.
7
8 640 pg TCDD-IEQ/g in potato, where we found 1.4-2.4 pg TCDD-IEQ/g (Table I).
9
10
11 However, based on our measurement with the 4h exposure time, we found in line with
12
13 Jeuken *et al.* a much higher amount of 110-332 pg TCDD-IEQ/g in potato. The
14
15 interpretation of DR-CALUX[®] measurements on extracts of food items is thus very
16
17 complicated, and we do not pretend to give a conclusive opinion about their eventual
18
19 toxic potential in the human diet. Important, however, is to obtain an estimation which
20
21 shows whether the exposure to NAhRAs is considerably higher than to xenobiotic AhR
22
23 agonists such as dioxins and other persistent compounds in the human diet, or much
24
25 lower and negligible. In 1999, the estimated intake of the sum of dioxins and dioxin-like
26
27 PCBs in The Netherlands was 1.2 pg WHO-TEQ per kg body weight (bw) per day,
28
29 which would be for a person weighing 70 kg 84 pg WHO-TEQ. Of this population was
30
31 8% exposed to intake levels above the tolerable weekly intake for dioxins and dioxin-
32
33 like PCBs of 14 pg WHO-TEQ per kg bw per week, or over 140 pg WHO-TEQ per
34
35 person per day. The average dietary intake of these xenobiotic AhR agonists in 1978
36
37 was nearly 700 pg WHO-TEQ per person per day (Baars and others 2004). These levels
38
39 are that far below the theoretical levels estimated by Jeuken *et al.* (e.g. 96000 pg
40
41 TCDD-IEQ per serving of 150 g potato), or our study (1545-6235 pg TCDD-IEQ/day
42
43 for part of the average daily diet), that the regular intake of NAhRAs should be
44
45 seriously evaluated with regard to the biological meaning of the level of AhR activation
46
47 that they may cause. On the one hand, the omnipresence of the competing natural AhR
48
49 agonists, together with the presence of many AhR antagonists (Amakura and others
50
51 2002; Amakura and others 2003; Baumgart and others 2005) in food, may appear as a
52
53
54
55
56
57
58
59
60

1
2
3
4 prophylaxis against xenobiotic intoxication via the AhR. On the other hand, several
5
6 human studies showed AhR responsive effects, like CYP1A2 induction, by several non-
7
8 dioxin like AhR agonists such as NAhRAs from cruciferous vegetables (Kall and others
9
10 1996; Murray and others 2001), I3C (Reed and others 2005), the anti-ulcer drug
11
12 omeprazole (Rost and others 1992), and heterocyclic aromatic amines (Sinha and others
13
14 1994). This seems to be in contrast with a regular exposure to numerous AhR agonists
15
16 in the human diet, which would constantly elevate the AhR activity. Another major
17
18 problem in this is, that the exact pathways by which the xenobiotic dioxins and dioxin-
19
20 like PCBs exert their toxicity are not well understood (Guzelian and others 2006;
21
22 Steenland and others 2004). This makes it very difficult to understand to what extent
23
24 NAhRAs, in amounts effective to cause AhR related effects, e.g. CYP1A2 induction by
25
26 concentrated food additives, also can initiate dioxin-like toxic pathways, especially
27
28 when slow processes like tumor promotion are involved. Besides tumor suppressing
29
30 capacity, high doses of NAhRAs given to laboratory animals have sometimes also
31
32 shown tumor promoting capacity (Dashwood 1998), and the accumulation effect of
33
34 persistent fat-soluble xenobiotic AhR agonists could be mimicked by constant or regular
35
36 NAhRA exposure.

37
38
39
40
41
42
43
44 In conclusion, this study confirms to a certain extent that the human diet contains a
45
46 large amount of AhR-activating substances of natural origin, which triggers questions
47
48 about the potential effects in humans, also in relation to the intake and adverse effects of
49
50 xenobiotic dioxins and dioxin-like PCBs. In order to elucidate the potential toxicity or
51
52 health-beneficial properties of these relatively large amounts of bioactive dietary
53
54 components, the toxic pathway(s) of dioxins in humans should be elucidated first. This
55
56 would give indications at what critical steps of the toxic pathway(s) the NAhRAs would
57
58
59
60

1
2
3
4 have a significant dioxin-like toxic effect or on the contrary, induce totally different or
5
6 even beneficial health effects, and what ultimately the predominant effect or the balance
7
8 of all these effects would be.
9
10

11 12 13 14 15 16 **Acknowledgement**

17
18 The authors thank Kyoko Sato, Liza Portier, Karin van Ede and Cornelis van de Kraats
19
20 kindly for their important contribution to the sample treatment and the DR CALUX[®]
21
22 analysis. The Dutch Centre of Human Nutrigenomics (CHN) supported this study.
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

References

- Aarts JM, Denison MS, Cox MA, Schalk MA, Garrison PM, Tullis K, de Haan LH, Brouwer A. 1995. Species-specific antagonism of Ah receptor action by 2,2',5,5'-tetrachloro- and 2,2',3,3',4,4'-hexachlorobiphenyl. *Eur J Pharmacol* 293(4):463-74.
- Amakura Y, Tsutsumi T, Nakamura M, Kitagawa H, Fujino J, Sasaki K, Yoshida T, Toyoda M. 2002. Preliminary screening of the inhibitory effect of food extracts on activation of the aryl hydrocarbon receptor induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Biol Pharm Bull* 25(2):272-4.
- Amakura Y, Tsutsumi T, Sasaki K, Yoshida T, Maitani T. 2003. Screening of the inhibitory effect of vegetable constituents on the aryl hydrocarbon receptor-mediated activity induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Biol Pharm Bull* 26(12):1754-60.
- Baars AJ, Bakker MI, Baumann RA, Boon PE, Freijer JI, Hoogenboom LA, Hoogerbrugge R, van Klaveren JD, Liem AK, Traag WA and others. 2004. Dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs: occurrence and dietary intake in The Netherlands. *Toxicol Lett* 151(1):51-61.
- Baumgart A, Schmidt M, Schmitz HJ, Schrenk D. 2005. Natural furocoumarins as inducers and inhibitors of cytochrome P450 1A1 in rat hepatocytes. *Biochem Pharmacol* 69(4):657-67.
- Bjeldanes LF, Kim JY, Grose KR, Bartholomew JC, Bradfield CA. 1991. Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Proc Natl Acad Sci U S A* 88(21):9543-7.
- Bovee TF, Hoogenboom LA, Hamers AR, Traag WA, Zuidema T, Aarts JM, Brouwer A, Kuiper HA. 1998. Validation and use of the CALUX-bioassay for the determination of dioxins and PCBs in bovine milk. *Food Addit Contam* 15(8):863-75.
- Chen YH, Riby J, Srivastava P, Bartholomew J, Denison M, Bjeldanes L. 1995. Regulation of CYP1A1 by indolo[3,2-b]carbazole in murine hepatoma cells. *J Biol Chem* 270(38):22548-55.
- Dashwood RH. 1998. Indole-3-carbinol: anticarcinogen or tumor promoter in brassica vegetables? *Chem Biol Interact* 110(1-2):1-5.
- Denison MS, Nagy SR. 2003. Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. *Annu Rev Pharmacol Toxicol* 43:309-34.
- Guzelian P, Quattrochi L, Karch N, Aylward L, Kaley R. 2006. Does dioxin exert toxic effects in humans at or near current background body levels?: An evidence-based conclusion. *Hum Exp Toxicol* 25(2):99-105.
- Hankinson O. 1995. The aryl hydrocarbon receptor complex. *Annu Rev Pharmacol Toxicol* 35:307-40.
- Hoogenboom L, Goeyens L, Carbonnelle S, van Loco J, Beernaert H, Baeyens W, Traag WA, Bovee TF, Jacobs G, Schoeters G. 2006a. The CALUX bioassay: Current status of its application to screening food and feed. *Trends in Analytical Chemistry* 25(4):410-420.
- Hoogenboom R, Bovee T, Traag W, Hoogerbrugge R, Baumann B, Portier L, van de Weg G, de Vries J. 2006b. The use of the DR CALUX bioassay and indicator

- 1
2
3
4 polychlorinated biphenyls for screening of elevated levels of dioxins and dioxin-
5 like polychlorinated biphenyls in eel. *Mol Nutr Food Res* 50(10):945-57.
6
7 Hulshof KFAM, Ocke MC, van Rossum CTM, Buurma-Rethans EJM, Brants HAM,
8 Drijvers JJMM, ter Doest D. 2004. Resultaten van de Voedselconsumptiepeiling
9 2003 (in Dutch). RIVM. Report nr 350030002/2004. 1-111 p.
10
11 Jeuken A, Keser BJG, Khan E, Brouwer A, Koeman J, Denison MS. 2003. Activation
12 of the Ah receptor by extracts of dietary herbal supplements, vegetables, and
13 fruits. *Journal of Agricultural and Food Chemistry* 51(18):5478-5487.
14
15 Kall MA, Vang O, Clausen J. 1996. Effects of dietary broccoli on human in vivo drug
16 metabolizing enzymes: evaluation of caffeine, oestrone and chlorzoxazone
17 metabolism. *Carcinogenesis* 17(4):793-9.
18
19 Kim YS, Milner JA. 2005. Targets for indole-3-carbinol in cancer prevention. *J Nutr*
20 *Biochem* 16(2):65-73.
21
22 Leibelt DA, Hedstrom OR, Fischer KA, Pereira CB, Williams DE. 2003. Evaluation of
23 chronic dietary exposure to indole-3-carbinol and absorption-enhanced 3,3'-
24 diindolylmethane in sprague-dawley rats. *Toxicol Sci* 74(1):10-21.
25
26 Machala M, Vondracek J, Blaha L, Ciganek M, Neca JV. 2001. Aryl hydrocarbon
27 receptor-mediated activity of mutagenic polycyclic aromatic hydrocarbons
28 determined using in vitro reporter gene assay. *Mutat Res* 497(1-2):49-62.
29
30 Murray S, Lake BG, Gray S, Edwards AJ, Springall C, Bowey EA, Williamson G,
31 Boobis AR, Gooderham NJ. 2001. Effect of cruciferous vegetable consumption
32 on heterocyclic aromatic amine metabolism in man. *Carcinogenesis* 22(9):1413-
33 20.
34
35 Nishiumi S, Yabushita Y, Fukuda I, Mukai R, Yoshida K, Ashida H. 2006. Molokhia
36 (*Corchorus olitorius* L.) extract suppresses transformation of the aryl
37 hydrocarbon receptor induced by dioxins. *Food Chem Toxicol* 44(2):250-60.
38
39 Pohjanvirta R, Korkalainen M, McGuire J, Simanainen U, Juvonen R, Tuomisto JT,
40 Unkila M, Viluksela M, Bergman J, Poellinger L and others. 2002. Comparison
41 of acute toxicities of indolo[3,2-b]carbazole (ICZ) and 2,3,7,8-
42 tetrachlorodibenzo-p-dioxin (TCDD) in TCDD-sensitive rats. *Food Chem*
43 *Toxicol* 40(7):1023-32.
44
45 Pohl C, Will F, Dietrich H, Schrenk D. 2006. Cytochrome P450 1A1 expression and
46 activity in Caco-2 cells: modulation by apple juice extract and certain apple
47 polyphenols. *J Agric Food Chem* 54(26):10262-8.
48
49 Reed GA, Peterson KS, Smith HJ, Gray JC, Sullivan DK, Mayo MS, Crowell JA,
50 Hurwitz A. 2005. A phase I study of indole-3-carbinol in women: tolerability
51 and effects. *Cancer Epidemiol Biomarkers Prev* 14(8):1953-60.
52
53 Rogan EG. 2006. The natural chemopreventive compound indole-3-carbinol: state of
54 the science. *In Vivo* 20(2):221-8.
55
56 Rost KL, Brosicke H, Brockmoller J, Scheffler M, Helge H, Roots I. 1992. Increase of
57 cytochrome P450IA2 activity by omeprazole: evidence by the 13C-[N-3-
58 methyl]-caffeine breath test in poor and extensive metabolizers of S-
59 mephenytoin. *Clin Pharmacol Ther* 52(2):170-80.
60
61 Safe S. 2001. Molecular biology of the Ah receptor and its role in carcinogenesis.
62 *Toxicology Letters* 120(1-3):1-7.
63
64 Safe SH. 1998. Development validation and problems with the toxic equivalency factor
65 approach for risk assessment of dioxins and related compounds. *J Anim Sci*
66 76(1):134-41.

- 1
2
3
4
5 Sinha R, Rothman N, Brown ED, Mark SD, Hoover RN, Caporaso NE, Levander OA,
6 Knize MG, Lang NP, Kadlubar FF. 1994. Pan-fried meat containing high levels
7 of heterocyclic aromatic amines but low levels of polycyclic aromatic
8 hydrocarbons induces cytochrome P4501A2 activity in humans. *Cancer Res*
9 54(23):6154-9.
- 10 Steenland K, Bertazzi P, Baccarelli A, Kogevinas M. 2004. Dioxin revisited:
11 developments since the 1997 IARC classification of dioxin as a human
12 carcinogen. *Environ Health Perspect* 112(13):1265-8.
- 13 Windal I, Denison MS, Birnbaum LS, Van Wouwe N, Baeyens W, Goeyens L. 2005.
14 Chemically activated luciferase gene expression (CALUX) cell bioassay
15 analysis for the estimation of dioxin-like activity: critical parameters of the
16 CALUX procedure that impact assay results. *Environ Sci Technol* 39(19):7357-
17 64.
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60