資　料　8

平成１９年度　食品・添加物等規格基準に関する試験検査等の実施について（規格基準関係）

食品中のかび毒に係る汚染実態調査

（ピーナッツトータルアフラトキシン実態調査）

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1. はじめに

我が国では、発がん性かび毒であるｱﾌﾗﾄｷｼﾝについては、全ての食品に対しｱﾌﾗﾄｷｼﾝB_1（AFB_1）が検出されてはならないことと規制している。

しかしながら、国際的には、アフラトキシンの基準値はトータルアフラトキシン（アフラトキシンB_1, B_2, G_1及びG_2）として設定される方向にあることから、我が国においても将来的にトータルアフラトキシンとして基準値が設定され得ることを想定し、調査研究が進められてきたところである。これまでの調査研究によると、アフラトキシンB群とG群が産生される場合は、AFB_1がその大半をしめていると考えられてきた。しかし近年ナッツ類などではB群よりG群の方が多く検出される例が報告されている。

ついては、今後我が国においてトータルアフラトキシンの基準値を検討する場合には、B群とG群の複合汚染の比率を考慮して基準値を策定する必要があることから、トータルアフラトキシンの汚染実態について詳細な調査を実施する。

また、先日、ECにおいて、アフラトキシンの新たな毒性評価等がなされたことから、最新の知見について情報収集を行う。

2. イムノアフィニティーカラムによるピーナッツ中のトータルアフラトキシン分析法の妥当性について

1）目的

トータルアフラトキシンの実態調査を行うにあたって、用いる分析法の妥当性を検討する必要があることから、国際的にもトータルアフラトキシンの実態調査においてよく用いられている、イムノアフィニティカラム(IAC)により前処理を行い高速液体クロマトグラフィー（HPLC）－蛍光検出法により測定を行う方法を用いて，検出法の妥当性を検討した。

2）材料と方法

(1) 試料

アフラトキシン無汚染ピーナッツを購入し、1mm以下に粉砕し、200gづつ袋詰めをし、試験に供した。

(2) 試薬

抽出溶媒として90%アセトニトリル水溶液を用いた。PBSは、PBS TABLETS(MP

Biomedicals 社製)1Tablet を 100ml に溶かして使用した。Tween20(ポリオキシエチレン(20)ソルビタンモノラウレート)は、Wako 社製、ICI 社商標 Tween20 相当品を用いた。トリフルオロ酢酸(TFA)は、SIGMA 社製のものを用い、アセトニトリルおよびメタノールは、LC グレードのものを用いた。ろ紙は(Grade 4)およびガラスフィルター(934-AH、Whatman 社)を用いた。イムノアフィニティーカラム(IAC)は、Aflaking(堀場製作所(株))を用いた。トータルアフラトキシン標準液は、4 種類のアフラトキシンをそれぞれ最終濃度 0.5μg/mL および 5.0 μg/mL になるようにアセトニトリルで溶解したものを調整し、用いた。

(3) 添加回収試験

室温に戻した試料 10.0g に、添加液を 100μl 添加し、一時間遮光静置した。その後、測定方法に準じて測定を行った。

(4) 分析方法

試料 10.0g に抽出溶媒を 40.0ml を入れ、5 分間ブレンドし抽出した。ろ紙でろ過した後、抽出液を PBS で 5 倍希釈し、ガラスフィルターでろ過した。ろ液 10.0ml を正確に量り IAC に負荷した。IAC の洗浄は、PBS 3.3ml×3 回、水 3.3ml ×3 回の順に流し洗浄した。IAC を洗浄した後、アセトニトリル 1.0ml×3 回で溶出し、窒素乾固した。0.1ml の TFA を入れ激しく撹拌し、15 分間遮光で反応させた。アセトニトリル･水(1:9)の混合液 0.4ml を入れ撹拌した後、フィルター(0.45μm)を通し、HPLC 用試験溶液とした。ブランクは、試料に添加液を添加せずに抽出し、IAC で同様に前処理をして HPLC 用試験溶液を作成した　(スキーム 1)。

（4）分析条件

分析条件は、平成１４年に出された通知法にしたがって以下の通り行った。

移動相　：アセトニトリル：メタノール：水（１：３：６）

カラム　：Inertsil ODS-2 4.6×150mm(5μm)

温　度　：40℃

流　速　：1.0mL/min

検出波長：Ex 365nm Em 450nm

注入量　：10μL

1）試料　10.0g+90%アセトニトリル 40mL

2）ワーニングブレンダー 10 分間

3）遠 心

4）上清から4.0mL とってリン酸緩衝液（PBS）または精製水で20.0mL とする

5）ガラス繊維ろ紙 Whatman934AH でろ過

6）IACに10mL 注入※滴下速度に注意

7）PBS 10mL（以上）洗浄

8）蒸留水 10mL（以上）洗浄

9）カラム中の水分を完全に出す ※注射筒で完全に押し出す

１０）アセトニトリル3.0mL 溶出

１１）窒素ガスで乾固

１２）TFA 0.1mLを加え反応

１３）室温暗所 15 分

１４）アセトニトリル：水（１：９） 0.4mL

１５）HPLCで分析

Sheme 1. ピーナッツ中のトータルアフラトキシン分析法妥当性試験　プロトコル

3）結果

妥当性試験は、（財）日本食品分析センター、（財）日本冷凍食品検査協会、（財）日本マイコトキシン検査協会の３機関でおこなった。

表 2-1 に示したように 0.5 ng/g および 5.0 ng/g を添加した場合、４種類のアフラトキシンにおいていずれも室内併行精度 (relative repeatability) 4.0% 以下で、室間再現精度 (relative reproducibility)も 14% 以下であり、HoRat 値が 0.3 以下であった。機関数が少ないことから HoRat 値はあまり評価の対象にはならないが、室内併行精度および室間再現精度は極めて良好であった。このことから、本試験法は、実態調査試験法として妥当であることが示めされた。

表2-1　　ピーナッツ中のトータルアフラトキシン分析法の妥当性の検討

	0.5ng/g添加	B1		B2		G1		G2	
	機関 1	0.47	0.46	0.52	0.49	0.40	0.43	0.46	0.46
	機関 2	0.517	0.524	0.513	0.522	0.447	0.479	0.457	0.489
	機関 3	0.53	0.53	0.52	0.52	0.54	0.54	0.52	0.53
Repeatability SD [Sr]		0.01		0.01		0.02		0.01	
Repeatability relative SD [RSDr,%]		1.04		2.52		3.79		2.82	
Repeatability value [r(2.8*Sr)]		0.01		0.04		0.05		0.04	
Reproducibility									
Reproducibility SD [SR]		0.03		0.01		0.06		0.04	
Reproducibility relative SD [RSDR,%]		6.86		2.27		13.61		7.35	
Reproducibility value [R(2.8*SR)]		0.10		0.03		0.18		0.10	
HORRAT		0.14		0.05		0.27		0.15	

	5,0ng/g添加	B1		B2		G1		G2	
	機関 1	5.03	5.17	5.27	5.52	5.34	5.58	5.62	5.95
	機関 2	5.06	5.22	5.15	5.34	5.49	5.69	5.42	5.71
	機関 3	4.70	4.80	4.58	4.56	4.80	4.82	4.69	4.71
Repeatability SD [Sr]		0.10		0.13		0.13		0.18	
Repeatability relative SD [RSDr,%]		1.92		2.54		2.39		3.38	
Repeatability value [r(2.8*Sr)]		0.27		0.36		0.35		0.51	
Reproducibility									
Reproducibility SD [SR]		0.23		0.45		0.43		0.59	
Reproducibility relative SD [RSDR,%]		4.53		8.86		8.08		10.98	
Reproducibility value [R(2.8*SR)]		0.63		1.26		1.20		1.64	
HORRAT		0.13		0.25		0.23		0.31	

４）考察

コーデックスにおいてピーナッツ中のトータルアフラトキシンに対して基準値が設定されていることから、分析法に関しては AOAC や CEN などに多くの方法が記載されている。その中において IAC を用いる方法は一般的である。ただし、国際的な方法の多くは抽出溶媒に 70% メタノール溶液を用いている。我が国では、通知法において多機能カラムを用いる方法を採用していることから、抽出溶媒に 90% アセトニトリル溶液を用いている。また、昨年の食品等試験検査費「食品中のかび毒に係る試験検査」において検討した結果、ピーナッツでは 90% アセトニトリル溶液の方が 70% メタノールより抽出効率が高いことが、添加回収試験および自然汚染ピーナッツを用いた試験においても確認されていることから、今回の実態調査では抽出溶媒として 90% アセトニトリル溶液を用いる方法を採用した。

国際的には、分析法の妥当性を評価する手段として複数機関によるコラボラティブスタディなどが用いられているが、本試験では３機関による検討を行った。表２－２に、EU の要求する分析法のクライテリアを示したが、本試験で得られた結果はいずれも回収率は 95%以上であり、このクライテリアを充分満たしていた。よって本プロトコールによる分析法は、ピーナッツ中のトータルアフラトキシンは分析法として妥当性があるものと評価できる。

表 2-2　EU の要求する分析法のクライテリア

(Commission Directive 98/53/EC of 16 July 1998)

基準	基準値	推奨する回収率	最大許容量
ブランク		陰性	
トータル	＜ 1.0 μg/L	50-120 %	
アフラトキシン	1-10.0μg/L	70-110 %	
室間変動の精度		ＨｏＲａｔの式に準じる。２．０	

3．市販ピーナッツ、アーモンド、くるみ、ヘーゼルナッツ、ピスタチオのトータルアフラトキシン汚染実態

（1）目的

近年ナッツ類などではAFB群よりAFG群の方が多く検出される例が報告されている。ついては、我が国において、トータルアフラトキシン汚染実態の詳細な調査を実施し、これらの分布について検討をおこなった。

（2）材料および方法

試料：実態調査に用いたピーナッツ試料は、全国の十数カ所の市町村の市販品から無作為に購入したもの１９２検体をトータルアフラトキシンの分析に供した。試料の詳細は参考として添付した。ピーナッツ以外に木の実としてアーモンド　３６検体、くるみ　８検体、ヘーゼルナッツ　７検体、ピスタチオ　９検体のトータルアフラトキシンを分析した。

分析法は、汚染のばらつきを考慮し試料量を50gとし、２で妥当性を検討した方法を用いておこなった（スキーム3-1）。　アーモンド、くるみ、ヘーゼルナッツ、ピスタチオも同様の方法により分析した。

（3）結果

(i) ピーナッツ

２機関において８０検体を、１機関において３２検体を分析した。それぞれの機関における回収率は、92.0～110％であり、検出限界は0.1～0.5 ng/gであった。バターピーナッツ１検体からトータルアフラトキシンで0.4 ng/gの汚染が認められたが，残り191検体は検出限界以下の汚染であった。汚染の内訳は、AFB_1　0.2 ng/g および AFG_1　0.2 ng/g であり、AFB_1 と AFG_1 が同等の汚染であった。

(ii) アーモンド

アーモンドは１機関で分析を行った。添加回収率は0.1 ng/gの添加で、AFB_1 117±8.9 %, AFB_2 109.5±1,5 %，AFG_1 79.6±5,5 % AFG_2 104,2±7.4 %であり，5.0 ng/gの添加で、AFB_1 94.2±3.7 %, AFB_2 91.6±2.6 %，AFG_1 75.8±7.9 %

AFG_2 88.0±0.3 %であった。アーモンドでは検出限界は0.01 ng/g,定量限界は0.1 ng/g, くるみ、ヘーゼルナッツ、ピスタチオでは検出限界は 0.04 ng/g, 定量限界は0.1 ng/gであった。

くるみ、ヘーゼルナッツからは，検出限界以上のトータルアフラトキシンは検出されなかったが、アーモンドでは３６検体中２４検体にトータルアフラトキシン汚染が検出され、そのうち２検体でBGグループであった。、ピスタチオは９検体中２検体にAFB_1, AFB_2の汚染が検出された。２検体ともBグループのみの汚染であった（表３－１、表３－２)。

（４）考察

我が国に流通しているピーナッツおよび木の実類のトータルアフラトキシンの汚染実態を検討したところ、現在のところ汚染はほとんどなかった。一部検出された検体もあったが、その汚染量は極めて低いレベルであった。しかしピーナッツで検出されたトータルアフラトキシンでは，AFB_1とAFG_1が同等のレベルで検出されていた事例があった。これはAFB_1が優位であった従来の傾向とは異なった傾向である。　アーモンド、ピスタチオでは、従来通りAFB_1が優位の汚染であった。

Total AF試験法(ピーナッツ実態調査)

フロー図	手順、条件	注解
試料調製	試料全量を均一に粉砕する	同一製品はすべて粉砕する 冷凍保存する
試料の秤量	50.0gを正確に秤量する	試料は室温にもどす
抽出	アセトニトリル-水(90:10)200mlを加える ホモゲナイザーまたはワーニングブレンダー等で5分間ブレンド	ホモゲナイザー、ワーニングブレンダーと同等品を使用
遠過または遠心分離	遠心または遠過(Whatman No.1またはNo.4)	遠心して油分が分離する場合は、遠心しないで遠過する。
抽出溶液	上清から4.0mLとって精製水で20.0mLとする	ろ液が濁っている場合は0.1-0.4%のTween20を使うことは可能
	ガラス繊維ろ紙 Whatman934AHでろ過	アフィニティカラムに負荷する液は澄んでいることが重要 アフィニティカラムはあらかじめ室温に戻し、ゲルの中に空気を入れないようにキャップをあけること
精製	アフィニティカラムに10mL負荷	自然落下でおこなう
	PBS 10mL(以上)洗浄	自然落下でおこなう 着色する場合は0.01-0.1%Tween 20 in PBSで洗浄
	蒸留水10mL(以上)洗浄	自然落下でおこなう この操作の時に空気を入れないことが重要
	カラム中の水分を完全に出す	シリンジなどを用いる
	アセトニトリル 3 mlで溶出させる	1ml入れて自然落下で溶出させた後5分間静置させる。さらに1ml加え溶出させ、もう一度繰り返す。
減圧乾固	40℃以下、窒素気流で減圧乾固	水分がのこらないように注意 バイアルはLC測定用 できればシラン加工バイアル瓶を使用
TFA反応	TFA 0.1 mlを残渣に直接加える	ボルテックスでよく撹拌する
	室温暗所で15分	
	H2O-CH3CN (90:10) 0.4mLで溶解 孔径0.2 μmメンブランフィルターで濾過 HPLCに供する	0.5g/0.5 ml
HPLC condition	LCカラム:ODS, 250x4.6mm, 3-5umを使用	
	カラム温度:40℃ 注入量:20-50uL 流速 1.0mL/min 検出波長: Ex 365nm Em 450nm 移動相:アセトニトリル:メタノール:水(1:3:6)	

Scheme 3-1 実態汚染調査用分析法

表３－１　アーモンドのトータルアフラトキシン汚染実態

検体番号	AFB1	AFB2	AFG1	AFG2	購入場所	原産国
1	ND	ND	ND	ND	東京	-
2	ND	ND	ND	ND	東京	アメリカ
3	ND	ND	ND	ND	東京	ｱﾒﾘｶ合衆国
4	ND	ND	ND	ND	東京	-
5	ND	ND	ND	ND	東京	米国産
6	ND	ND	ND	ND	東京	-
7	0.03	ND	ND	ND	兵庫	-
8	0.09	ND	ND	ND	兵庫	-
9	0.11	0.02	0.02	trace	兵庫	-
10	0.11	ND	ND	ND	兵庫	-
11	0.01	ND	ND	ND	群馬	
12	trace	ND	ND	ND	群馬	
13	0.09	0.01	0.03	0.01	群馬	
14	0.04	ND	ND	ND	群馬	
15	0.05	ND	ND	ND	群馬	
16	0.05	ND	ND	ND	群馬	
17	ND	ND	ND	ND	群馬	
18	0.02	0.01	ND	ND	群馬	
19	ND	ND	ND	ND	群馬	アメリカ
20	ND	ND	ND	ND	群馬	アメリカ
21	0.06	ND	ND	ND	群馬	
22	ND	ND	ND	ND	群馬	
23	ND	ND	ND	ND	群馬	
24	0.01	ND	ND	ND	東京	-
25	trace	ND	ND	ND	東京	-
26	ND	ND	ND	ND	東京	-
27	0.06	0.01	ND	ND	東京	ｶﾘﾌｫﾙﾆｱ
28	0.01	ND	ND	ND	東京	-
29	0.01	ND	ND	ND	東京	アメリカ
30	0.01	ND	ND	ND	東京	アメリカ
31	0.01	ND	ND	ND	東京	アメリカ
32	0.03	ND	ND	ND	東京	不明
33	0.03	0.02	ND	ND	東京	-
34	0.03	ND	ND	ND	東京	不明
35	0.01	ND	ND	ND	東京	不明
36	0.03	trace	ND	ND	東京	

検出限界 0.01ng/g　　定量限界 0.1 ng/g

表３－２　くるみ、ヘーゼルナッツ、ピスタチオの汚染実態

くるみ　　ng/g

検体番号	AFB1	AFB2	AFG1	AFG2	購入場所	原産国
1	ND	ND	ND	ND	東京	アメリカ
2	ND	ND	ND	ND	東京	ー
3	ND	ND	ND	ND	東京	ー
4	ND	ND	ND	ND	東京	アメリカ
5	ND	ND	ND	ND	東京	中国
6	ND	ND	ND	ND	東京	アメリカ産
7	ND	ND	ND	ND	東京	アメリカ
8	ND	ND	ND	ND	東京	ー

ヘーゼルナッツ

検体番号	AFB1	AFB2	AFG1	AFG2	購入場所	原産国
1	ND	ND	ND	ND	東京	米国
2	ND	ND	ND	ND	東京	トルコ
3	ND	ND	ND	ND	東京	トルコ
4	ND	ND	ND	ND	東京	不明
5	ND	ND	ND	ND	東京	アメリカ
6	ND	ND	ND	ND	東京	アメリカ
7	ND	ND	ND	ND	東京	トルコ

ピスタチオ

検体番号	AFB1	AFB2	AFG1	AFG2	購入場所	原産国
1	ND	ND	ND	ND	東京	イラン
2	0.71	0.06	ND	ND	東京	イラン
3	ND	ND	ND	ND	東京	アメリカ
4	ND	ND	ND	ND	東京	アメリカ
5	ND	ND	ND	ND	東京	米国
6	ND	ND	ND	ND	東京	アメリカ
7	ND	ND	ND	ND	東京	イラン
8	0.3	ND	ND	ND	東京	アメリカ
9	ND	ND	ND	ND	東京	イタリア

LOQ　0.1ng/g　　LOD　0.04ng/g

4．命令検査結果からのピーナッツ中のトータルアフラトキシン汚染分布の変遷

1）目的

ピーナッツおよび木の実の実態調査からは、現時点ではアフラトキシンGグループが極端に高いものが市場に出回っている可能性は低いと考えられたが、我が国に輸入されているピーナッツにおいて、その汚染の頻度、各アフラトキシンの割合等の情報を把握することは今後の施策を策定する上で重要である。そのため、命令検査となっている輸入ピーナッツに関してのアフラトキシン検査結果をもとに汚染分布の変遷の解析を試みた。

2）材料および方法

命令検査となっているピーナッツに関しての、２００２年から２００６年までのアフラトキシン検査結果は、(財）日本マイコトキシン検査協会より提供された。１９７２年から１９８９年までの検査結果は、前田らの報告(Proc. Jpn. Assoc. Mycotoxicol., 31, 7-17, 1990)を参照とした。

分析法は２００２年から２００６年までの結果は通知法を用いており、１９７２年から１９８９年までの結果は、その当時の通知法である環食第１２８号で行っている。検出限界は、現通知法は各アフラトキシン0.1ng/gであり、環食第１２８号は10 ng/gである。

3）結果

（1）輸入ピーナッツの検査検体数

１９７２年から１９８９年までの輸入ピーナッツは小粒が主流であり、その主要輸入国は、アメリカであった。しかし２００２年時点では中国からの大型ピーナッツが主流となっている。小粒においてもアメリカからの輸入は年々減少傾向にあり、中国および南アフリカ殻の輸入が多くなってきている（表４－１）。

表　4-1　ピーナッツの主要輸入国と命令検査検体数

Year	China (Large type)	China (Small)	S.Africa	U.S.A
1972-1989	none	112	159	450
2002	1,328	386	378	298
2003	1,814	550	449	262
2004	1,683	621	207	170
2005	1,428	590	298	137
2006	1,645	576	252	138

（２）輸入ピーナッツにおけるアフラトキシン検出率

食品に汚染事例が多いアフラトキシン（AFB_1, AFB_2, AFG_1, AFG_2）の４つを総称してトータルアフラトキシンというが、汚染するカビの種類によって、B グループ（AFB_1 と AFB_2 のみが汚染しているもの）と BG グループ（４つすべて汚染しているもの）に分類される。

各輸入国からのピーナッツにおけるアフラトキシン検出率を表４-２に示した。各年のアフラトキシン汚染頻度は、収穫される年によってその汚染率は変化があるが、全体的に輸入量の１％程度に検出限界以上のアフラトキシンが検出されており、B グループ、BG グループとも汚染が認められている。B グループと BG グループの汚染比率は年ごとに異なっている。しかし、全体的に BG グループの汚染率は年々高くなる傾向が見られた（図４-１）。

表４－２　検査命令ピーナッツにおけるアフラトキシン検出率

		アフラトキシン検出率	
	Year	B group (%)	BG group(%)
China (Large)	2002	0.1	0.2
	2003	0.4	0.4
	2004	1	0.8
	2005	0.6	0.4
	2006	0.9	0.7
China (Small)	2002	0.5	0.0
	2003	0.4	0.2
	2004	0.2	0.3
	2005	0.3	0.2
	2006	0.3	0.3
S.Africa	2002	1.6	0.3
	2003	1.3	0.7
	2004	0.5	1
	2005	1.3	1
	2006	0.8	1.2
U.S.A	2002	1.7	0.3
	2003	6.2	0.8
	2004	0.6	0
	2005	2.2	2.2
	2006	4.3	1.4

岡野清志他　Mycotoxins, 58(2), in press (2008)

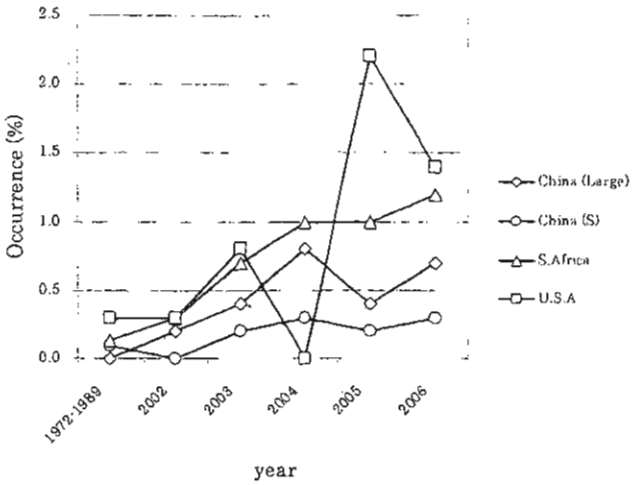

図４－１　命令検査ピーナッツにおけるアフラトキシンBGグループ汚染頻度

（３）アフラトキシン汚染輸入ピーナッツにおける各アフラトキシンの比率

BGグループの汚染が増加傾向があることが認められたが、その汚染ピーナッツ中の各アフラトキシンの比率を表４－３および図４－２～４－４に示した。

表４－３は中国から輸入された大粒のピーナッツでの比率であるが、2005年分はややAFB_1が高い比率を示してはいたが、2002年から2004年および2006年においてはAFB_1が20%以下、AFG_1が63%となっている。

表４－３　命令検査においてアフラトキシンが検出されたChina (Large type)の割合と各アフラトキシンの比率

	percentage of each aflatoxin (%)			
Year	AFB_1	AFB_2	AFG_1	AFG_2
2002	15.6	0.0	69.1	15.3
2003	14.1	3.1	66.8	16.0
2004	18.5	2.5	63.9	15.1
2005	39.3	6.2	41.5	13.0
2006	16.4	2.8	65.7	15.1

図４－２～４－４は，各輸入国別の年代別に見た小粒ピーナッツの各アフラトキシンの比率の変化を示したものであるが、1972-1989 年の汚染比率と比較して、中国とアメリカからの輸入ピーナッツでは 2002 年から劇的に AFG_1 の比率が高くなっている。南アフリカから輸入されたピーナッツでは、年によって AFB_1 の比率が高くなる傾向も見られるが 2003 年、2006 年では AFG_1 の比率が高くなっている。

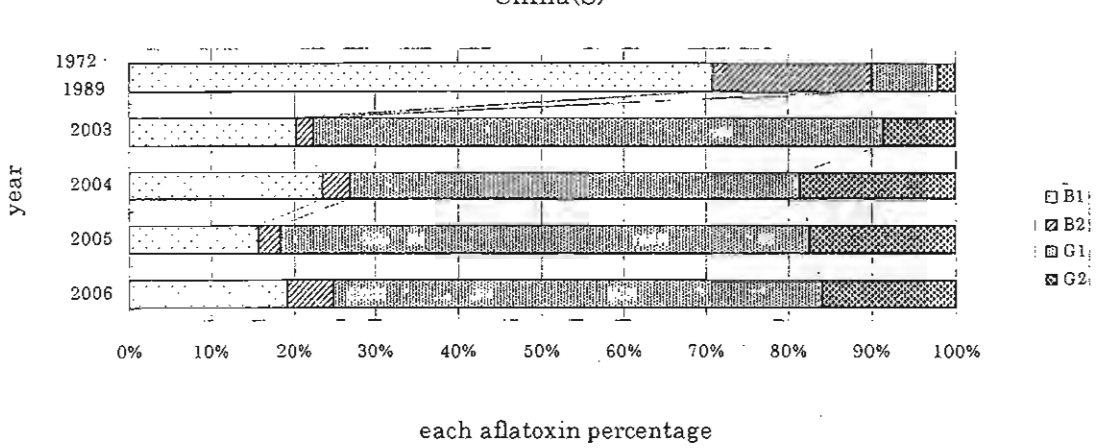

図４－２　中国から輸入された小粒ピーナッツの各アフラトキシン比率

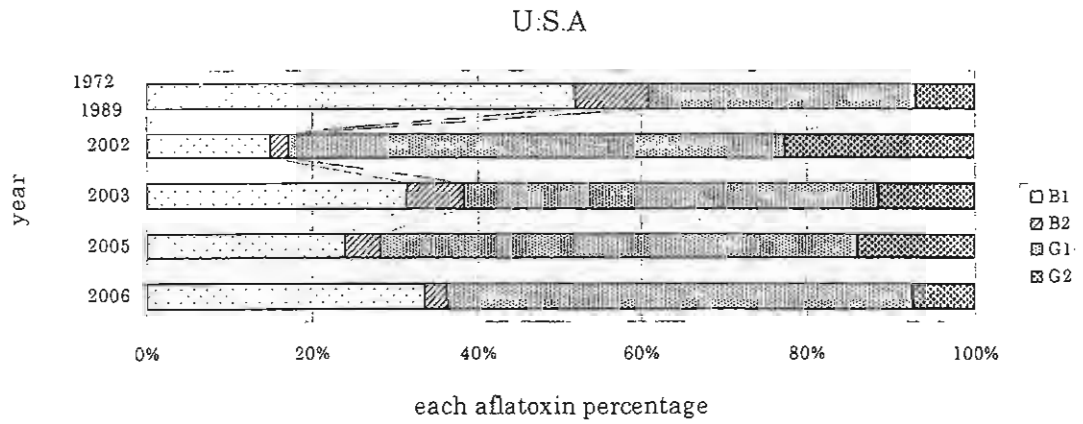

図４－３　アメリカから輸入された小粒ピーナッツの各アフラトキシン比率

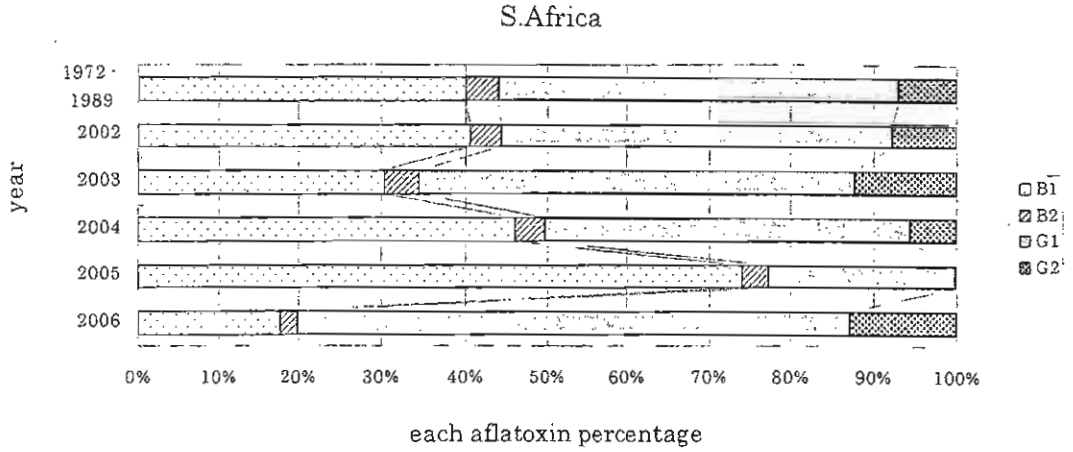

図４－４　南アフリカから輸入された小粒ピーナッツの各アフラトキシン比率

４．考察

ピーナッツは，輸入時のモニタリングで AFB_1 の汚染が多く検出されることから命令検査対象となっている。そのため、指定検査機関の検査が行われており、AFB_1 が規制値以上であるロットは市場には流通しない。　今回行った市販ピーナッツの汚染実態調査では、ほとんどのピーナッツにおいて検出限界以下であったが、それはこの検査システムが十分機能しているためと考えられる。しかし、輸入時の汚染状況を常に把握することは、今後の適切な行政施策策定に重要な知見となる。

そこで、命令検査に供されたピーナッツのデータを 1972 年から 1989 年までのものと 2002 年から 2006 年までのもので比較検討を行った。

1972 年から 1989 年までは、大粒のピーナッツはほとんど輸入されていなかった。そのため、この比較は小粒のピーナッツに限られるが、主要輸入国がアメリカから中国に変わったこと、両国におけるピーナッツの各アフラトキシン汚染比率が劇的に変化したことなどが見いだされた。大粒ピーナッツにおいては、AFB_1 より AFG_1 汚染が高い傾向が見いだされた。

これらの変化の原因は、いくつか考えられるが、その一つに、生産地の変化が考えられる。　アフラトキシン生産菌は、土壌や気候に影響を受けやすいため、生産地によって大きくその生息分布が異なる。一戸らは 2002 年から 2006

年までに検査されたピーナッツから、AFG_1汚染が高いものを選び、その菌を分離同定しているが、生産菌として *Aspergillus parasiticus* および *A. flavus* 近縁菌を指摘している(Mycotoxins, 58(2), in press (2008))。

2003年にFAOから出版された World regulations for mycotoxins in food and feed in 2003によれば、トータルアフラトキシン中、AFB1 と AFB2+AFG1+AFG2 の占める割合は1:0.8と記されている。また、昨年ECで行われたトータルアフラトキシンのリスクアナリシスのEFSAによる報告書では、AFB1のトータルアフラトキシンに占める割合は1/2と仮定している。現在多くの国でアフラトキシンに対する規制が設定されているが、ほとんどの国ではトータルアフラトキシンとして規制を作成しているため、AFB_1とその他のアフラトキシンの比率の変化はあまり大きな問題とはならない。しかし、我が国ではAFB1のみで規制していることから、AFG1の高い食品が流通する恐れがでてくる。

毒性的には、duckingに対するLD 50 (mg/kg)で比較すると、AFB_1 (0.3-0.4) =AFM_1(0.32)>AFG_1(0.78)>AFM_2(1.23)>AFB_2(1.7)>AFG_2(3.45)となり、AFG_1の毒性はAFB_1の半分と考えられる (Ciegler, A. Handbook of Microbiology, Vol.3, Microbial Products, p 525 (1973), CRC Press, Boca Raton, Florida)。また、AFG_1はAFB_1と同様発ガン機序に関わるフランの二重構造を有しており、実験動物の発ガン実験の結果から、AFB_1の10分の1程度の発がん性を有していると推測されている (IARC, 2002)。

これらの結果より、輸入ピーナッツの各アフラトキシン汚染分布の状況は変化するものであり、我が国のアフラトキシンによる健康被害を未然に防ぐために、トータルアフラトキシンとして規制を設定することが望まれる。

5. European Food Safety Authority:Opinion of the scitific panel CONTAM related to the potential increase of consumer health risk by a possible increase of the exicting maximum levels for aflatoxins in almonds, hazelnuts and pistachios and derived productspublication Date , 1 March 2007) の部分和訳

食品汚染の毒性学的評価および食品摂取の観点からの評価

食品汚染物質

アフラトキシン

(ピスタチオ、ヘーゼルナッツ、アーモンド、ブラジルナッツ、乾燥イチジクからの摂取評価、それぞれの Maximum level の影響)

The Joint FAO/WHO Expert Committee は、生産国より提出されるデータに基づき、アフラトキシン暴露におけるそれぞれの Maximum level (ML) の影響の評価を基礎データとすることを決定した。このことは、より上質なものが貿易上の原料であることを示し、また健常者におけるツリーナッツからのアフラトキシン暴露の評価結果となることを意味する。

アーモンド、ブラジルナッツ、ヘーゼルナッツ、ピスタチオ、乾燥イチジクの消費は１３地域の GEMS/Food クラスター群のうちの５地域において、食品からのトータルアフラトキシン (AFT) の暴露の 5%以上に寄与している。ヘーゼルナッツ、アーモンド、ピスタチオ、ブラジルナッツ、そしてドライフルーツ 1 kg あたり 20 μg の ML を完全に施行した場合、ツリーナッツを多く摂る消費者を含め、これらクラスターにおける食品からのアフラトキシンの暴露においてのみ相対的に寄与する影響を持つであろう。この寄与はピスタチオにおける高いアフラトキシン汚染にのみ由来する。ピスタチオ以外のツリーナッツにおいては、ML の存在はアフラトキシンの食品からの暴露において影響は持たない。さらに同委員会は ML 20 μg/kg に設定した場合と比較し、最も高い暴露にさらされている集団グループ５つのグループ全ての食品からの全体的な

AFTの暴露においてMLを15、10、8、4 μg/kgに設定することによる大きな影響はないであろうと結論づけている。
乾燥イチジクについては、同委員会はいかなる高水準のMLシナリオ（0, 4, 8, 10, 15, 20 μg/kg）を適用してもアフラトキシンの食品全体からの暴露における影響はないであろうと結論付けている。
同委員会はアフラトキシンの食品からの暴露を抑制することは、アフラトキシンに汚染されている可能性のある食品を高頻度に消費する集団に対して特に重要な公衆衛生上の目標であると指摘している。

アーモンド、ヘーゼルナッツ、ピスタチオとそれら加工品におけるアフラトキシンの現行のMLの可能な引き上げによる、消費者の健康リスクの潜在的な上昇にかかわる本委員会の依頼に対するCONTAMパネルの意見

2007年1月25日承認

Summary
アフラトキシンは熱帯湿潤な地域において多くみられるカビにより産生される。アフラトキシンの汚染は、ツリーナッツ、グラウンドナッツ、イチジクおよび他の乾燥果物、スパイス、混合植物油、ココア豆、トウモロコシに多く認められる。アフラトキシンは遺伝毒性および発ガン毒性を有すると考えられているため、リスクのない摂取量を設定することが不可能であり、1998年EUでは合理的に達成可能と考えられる範囲で限りなく低く抑えたレベルでアフラトキシンの規制を導入している。コーデックス規格は最近の議論において、EUにおいて近年施行された濃度より高濃度で、未加工のアーモンド・ヘーゼルナッツ・

ピスタチオにおけるトータルアフラトキシンのMaximum level（ML）を全世界的に設定することを提案している。その結果、Contaminants in the Food chain （CONTAM）のサイエンティフィックパネルは、EUにおけるこれらナッツ類の消費状況と、他の食品からのアフラトキシンの摂取を勘案し、アーモンド・ヘーゼルナッツ・ピスタチオにおけるトータルアフラトキシン（アフラトキシンB1, B2, G1, G2の総計）のEUのMLを 4 μg/kgから8もしくは10 μg/kg に変更を提案することによる消費者の健康へのリスクの増大の可能性を勧告することを求められている。

約４万におよぶ様々な食料品におけるアフラトキシン検査結果をCONTAMパネルは検討した。アフラトキシンは、検査されたサンプルのうち約75%からは検出されなかった。仮に存在していても、それぞれ異なる方法においての検出限界以下のアフラトキシン量であった。アフラトキシンが検出されるサンプルでは、アフラトキシンB1がトータルアフラトキシンにおいて一般的には主要な毒素であった。控えめな試算によると、CONTAM パネルはトータルアフラトキシンはアフラトキシンＢ１レベルの最大２倍であろうと推測している。またCONTAMパネルは業務用ミルクサンプル中のアフラトキシンＭ１(アフラトキシンB1の主要な代謝物)の濃度に関連するデータを入手していた。これらデータからアフラトキシンＭ１の汚染濃度は0.05 μg/kg以下であり、M1の低い発がん性の可能性を考慮し同パネルはこれらデータの更なる検討は実施しなかった。

アーモンド・ヘーゼルナッツ・ピスタチオに対するMLを可変できる範囲で変更した場合の影響を評価するため、CONTAMパネルはそれぞれ4、8、および10 μg/kg以上の汚染を排除した場合の食品からの暴露を見積もった。これら計算からトータルアフラトキシンのMLを4から8もしくは10 μg/kgに増加することにより、アーモンド・ヘーゼルナッツ・ピスタチオによるトータルアフラトキシンの暴露をわずかではあるが明確に上昇させうるが、平均濃度は提出された結果のテストでは1 μg/kg以下のままであろうことが示された。

アフラトキシンの汚染の潜在的な増加の影響の評価にはこれら三種類のナッツと他の食品からの摂取の両方を考慮する必要がある。全てのメンバー国の典型的な他の食品からの暴露にたいする健常者のデータを CONTAM パネルは利用できない。
数カ国の利用可能な食品からの暴露データの評価は、ヨーロッパの食品中における合理的な概算が GEMS/Food consumption Cluster Diets のデータベースから得られるため、CONTAM パネルはアーモンド・ヘーゼルナッツ・ピスタチオ以外の食品からのアフラトキシン群の食品からの暴露量の評価にこれらデータを利用している。これらナッツによる寄与はアフラトキシンの全食品からの暴露におけるわずか数%に過ぎなかった。

これら評価は、アーモンド・ヘーゼルナッツ・ピスタチオからのトータルアフラトキシンの ML を 4 から 8 もしくは 10 μg/kg への増加することで、全食品からのアフラトキシンの暴露の平均を 1%内で増加させることと結論している。

アーモンド・ヘーゼルナッツ・ピスタチオの消費データは、メンバー国のうち数カ国から利用可能であり、全て制約を受けた。それゆえ、CONTAM パネルはそれぞれのナッツについて最も低い消費のメンバー国から最も消費量の多いメンバー国までの、消費レベルの高い消費者の起こりうる暴露量データの範囲を推定した。これらの評価により、最も消費量の多い消費者においてトータルアフラトキシンに対する ML を 4 から 8 もしくは 10 μg/kg への増加させることで、全食品からのアフラトキシンの暴露を 20%まで増加しうることが示された。もし、期待されたように、ML を超えたナッツが時々消費された場合、食品から暴露の長期の平均は全体として高めとなるであろう。しかし、これら 3 種類のナッツの ML を 4 から 8 もしくは 10 μg/kg へ増加させた場合の相対的な影響は比較的低いであろう。

子供における食品からの暴露の概算は、成人に対する評価の範囲内であった。しかしこれらはナッツ以外の他の食品からの暴露を優位にしており、子供の食事特異的なデータのため利用は出来なかった。

ヘーゼルナッツとピスタチオにおける輸出前のコントロールにおける利用可能なデータは、トータルアフラトキシンのMLを4から8もしくは10μg/kgへ増加させることでEUマーケットにおいてナッツの6%の追加のバッチを許容するであろうことを示した。アーモンドに関しては利用可能な輸出前データは存在しない。

疫学的研究から肝臓がんのリスクファクターである慢性B型肝炎ウイルスの保菌者が高い地域においてアフラトキシンの暴露と肝細胞の発ガン頻度には明確な関連が指摘されている。CONTAM パネルは、発ガンにおいて多くの不確実性を表明している。最も感受性の高い齧歯類の系統と、慢性B型肝炎ウイルスの保菌者が、高頻度で、また非常に高いレベルでアフラトキシンに暴露されていると概算した一つのデータに由来するヒトのデータの妥当性を、格別に考慮してのことである。この研究では、研究された他の集団より肝臓がんのリスクが極めて高いことが示されていた。CONTAM パネルはこれらいかなるデータも無視することはでず、従って、これら全てのアセスメントにおける概算された食品からの暴露量を比較した。

The Joint FAO/WHO Expert Committee on Food Additives（JECFA）により有効的に行われた評価を基礎にし、またアフラトキシンの食品からの暴露の予想平均および高レベル暴露量を適用した場合の発ガンリスクの評価は、報告されたヨーロッパにおける肝細胞の発ガン率よりも少なくとも2オーダーレベル程度低い。このことはアフラトキシンがEUにおいては肝細胞の発ガンにおいて主要な因子ではないことを示唆している。

95%のベンチマーク用量信頼下限値と比較して BMDL10 における発ガン率は動物実験で 10%上昇することを基に、推定される全ての摂取量においてCONTAM パネルにより計算された暴露マージンは、ヒトの健康に有意に関連があることを示した。慢性B型肝炎ウイルスの感染率が高頻度で最も感受性の高いサブグループを含む集団からのデータを基に概算された BMDL10 と BMDL1 の値は、最も感受性の高いラットの系統におけるものと同レベルの感受性を示した。しかし他のサブグループはアフラトキシンの影響に対する感受

性はより低いと考えられた

CONTAM パネルは、アーモンド・ヘーゼルナッツ・ピスタチオにおけるトータルアフラトキシンの ML を 4 から 8 もしくは 10 μg/kg に変更することによる概算される食品からの暴露量と発ガンリスクおよび算出された暴露マージンへの影響は軽微であると結論付けた。

CONTAM パネルは、アフラトキシンは遺伝毒性と発ガン毒性を有していることから、全ての食品からのアフラトキシンの暴露は合理的に達成可能な範囲で出来る限り低濃度であるべきと結論付けた。このデータは、全食品からのアフラトキシンの暴露を抑制することは、市場に到達する高度に汚染された食品数を減らし、アーモンド・ヘーゼルナッツ・ピスタチオ以外の食品からの暴露を抑制することで達成可能であることを示している。

KEY WORDS
アフラトキシン群、アフラトキシン B1、アーモンド、ヘーゼルナッツ、ピスタチオ、暴露評価、肝細胞ガン、肝臓ガン、リスク評価、暴露マージン

6. まとめ

アフラトキシンは、発ガン性の強いカビ毒であるため、食品衛生上その制御は重大な課題である。アフラトキシンをはじめ多くのカビ毒は、気象条件に大きく影響を受けるため、汚染をゼロベースにすることは不可能である。また低分子で耐熱性を有するため、調理中の減毒化もあまり期待できない。そのため、多くの国では基準値等を設定して、暴露量の低減に努力している。

2003 年までにトータルアフラトキシンとして規制している国は 76 か国にのぼる。我が国は昭和 46 年に、現在の食品衛生法第 6 条により AFB1 のみ規制を設定し、以来その時点で最も感度が高い分析法の検出限界(10 ng/g)を実質的な基準値をして全食品を対象に適応している。いままでの多くの報告から、AFB_1 の汚染濃度は AFB_2, AFG_1, AFG_2 の合計した濃度より高いと考えられているので (FAO:World regulations for mycotoxins in food and feed in 2003)、我が国の規制の設定は妥当であるといえる。

しかし、平成 16 年度から 18 年度にかけて厚生労働科学研究事業において実施したトータルアフラトキシンの汚染実態調査と暴露評価の結果、市販ピーナッツ 150 検体のうち 1 検体からトータルアフラトキシン総量が 20ng/g 以上が検出された。この内訳を見ると、AFB_1 は規制値の 10 ng/g 以下であったが、AFG1 が 20ng/g 以上であった。この事実から、1) 市販のピーナッツで同様の現象が頻度高く検出されるかどうか 2) 現時点での輸入ピーナッツにおける トータルアフラトキシンにおけて AFB_1 が優位であるかが危惧された。

そのため本試験調査において、市販ピーナッツ、アーモンド、くるみ、ヘーゼルナッツ、ピスタチオのトータルアフラトキシン汚染実態および命令検査結果からのピーナッツ中のトータルアフラトキシン汚染変化の解析を行った。なお、市販ピーナッツ、アーモンド、くるみ、ヘーゼルナッツ、ピスタチオの実態調査に用いる分析法はあらかじめその妥当性を検証した。

全国から無作為に購入した市販ピーナッツ 192 検体を分析した結果、そのうち 1 検体から微量であるがＢＧグループ汚染が検出され、その汚染濃度比率は AFB_1 と AFG_1 で同等であった。アーモンド 36 検体中 2 検体からも微量であるが BG グループの汚染が検出されたが、その AFB_1 汚染濃度比率は、他の 3 種類の汚染濃度に比べ優位であった。これらの結果から、現時点では、厚生労働科学

研究事業において行った実態調査で認められたような汚染形態を有するピーナッツは、それほど高い頻度では流通していないことが確認された。

ピーナッツは輸入食品として命令検査の対象となっていることから、この検査結果を検討することによって輸入品の汚染実態を知ることができる。日本マイコトキシン検査協会は1972年からピーナッツの命令検査を行っており、そのデーターを有している。そこで当協会の協力を得て1972年から1989年までのトータルアフラトキシンの汚染状況と、2002年から2006年までの状況を比較し、各アフラトキシンの汚染比率の変遷を解析した。その結果、1）主要な輸入国はアメリカから中国になったこと。2）現在は中国産の大粒ピーナッツが主流であること。　3）大粒ピーナッツのアフラトキシン汚染比率はAFG_1の方がAFB_1より高いこと。4）小粒ピーナッツのアフラトキシン汚染比率は中国産、アメリカ産とも1972年から1989年までのデーターと比べるとAFG_1の比率が高くなっていることが明らかとなった。

最後に最新の情報として European Food Safety Authority:Opinion of the Scitific panel CONTAM が出した報告書の要旨の和訳を記した。

木の実のトータルアフラトキシン基準値設定の根拠となる暴露評価に関してCodex から JECFA へ諮問されたが、本報告書は JECFA での会議の前にヨーロッパにおける見解を示したものである。CONTAM パネルは、アーモンド・ヘーゼルナッツ・ピスタチオにおけるトータルアフラトキシンの Maximum level を 4 から 8 もしくは 10 μg/kg に変更することによる概算される食品からの暴露量と発ガンリスクおよび算出された暴露マージンへの影響は軽微であると結論付けた

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（財）マイコトキシン検査協会

（財）日本冷凍食品検査協会

平成１９年度

食品・添加物等規格基準に関する試験検査等の実施について

食品のかび毒に係る汚染実態調査

機　関　名　　財団法人　日本食品分析センター

研究責任者名（契約者）　理事長　齋藤　文一　㊞

食品のかび毒に係る汚染実態調査

1　依 頼 者

国立医薬品食品衛生研究所

2　検　　体

豆類　全80検体

3　試験概要

検体のトータルアフラトキシン量(アフラトキシンB_1, B_2, G_1及びG_2)をアフィニティカラムにより前処理を行い，分析した。

4　試験結果

分析結果を表-1に，添加回収試験の結果を表-2に，検出限界及び定量限界の算出結果を表-3にまとめた。また，表中でアフラトキシンは「AF」と略した。

なお，表-3から，検出限界を標準偏差の3倍，定量限界を10倍とすると，各アフラトキシンは定量限界0.5 ng/gの定量が可能であると判断された。

表-1-1　結果

検体番号	検体名称	結果(ng/g)			
		AFB_1	AFB_2	AFG_1	AFG_2
1	3-1(宮城)	<0.5	<0.5	<0.5	<0.5
2	3-2(宮城)	<0.5	<0.5	<0.5	<0.5
3	3-3(宮城)	<0.5	<0.5	<0.5	<0.5
4	4-1(秋田)	<0.5	<0.5	<0.5	<0.5
5	4-2(秋田)	<0.5	<0.5	<0.5	<0.5
6	4-3(秋田)	<0.5	<0.5	<0.5	<0.5
7	4-4(秋田)	<0.5	<0.5	<0.5	<0.5
8	4-5(秋田)	<0.5	<0.5	<0.5	<0.5
9	4-6(秋田)	<0.5	<0.5	<0.5	<0.5
10	4-7(秋田)	<0.5	<0.5	<0.5	<0.5
11	4-8(秋田)	<0.5	<0.5	<0.5	<0.5
12	4-9(秋田)	<0.5	<0.5	<0.5	<0.5
13	13-20(群馬)	<0.5	<0.5	<0.5	<0.5
14	13-21(群馬)	<0.5	<0.5	<0.5	<0.5
15	13-22(群馬)	<0.5	<0.5	<0.5	<0.5
16	13-23(群馬)	<0.5	<0.5	<0.5	<0.5
17	13-25(群馬)	<0.5	<0.5	<0.5	<0.5
18	22-25(静岡)	<0.5	<0.5	<0.5	<0.5
19	22-26(静岡)	<0.5	<0.5	<0.5	<0.5
20	22-27(静岡)	<0.5	<0.5	<0.5	<0.5
21	32-1(岡山)	<0.5	<0.5	<0.5	<0.5
22	32-2(岡山)	<0.5	<0.5	<0.5	<0.5
23	32-3(岡山)	<0.5	<0.5	<0.5	<0.5
24	32-4(岡山)	<0.5	<0.5	<0.5	<0.5
25	32-6(岡山)	<0.5	<0.5	<0.5	<0.5
26	33-2(広島)	<0.5	<0.5	<0.5	<0.5
27	33-3(広島)	<0.5	<0.5	<0.5	<0.5
28	34-2(山口)	<0.5	<0.5	<0.5	<0.5
29	34-3(山口)	<0.5	<0.5	<0.5	<0.5
30	37-1(愛媛)	<0.5	<0.5	<0.5	<0.5
31	37-2(愛媛)	<0.5	<0.5	<0.5	<0.5
32	39-1(福岡)	<0.5	<0.5	<0.5	<0.5
33	39-2(福岡)	<0.5	<0.5	<0.5	<0.5
34	39-3(福岡)	<0.5	<0.5	<0.5	<0.5
35	39-4(福岡)	<0.5	<0.5	<0.5	<0.5
36	39-5(福岡)	<0.5	<0.5	<0.5	<0.5
37	39-6(福岡)	<0.5	<0.5	<0.5	<0.5
38	39-7(福岡)	<0.5	<0.5	<0.5	<0.5
39	39-8(福岡)	<0.5	<0.5	<0.5	<0.5
40	39-9(福岡)	<0.5	<0.5	<0.5	<0.5

表-1-2 結果

検体番号	検体名称	結果(ng/g)			
		AFB_1	AFB_2	AFG_1	AFG_2
41	39-10(福岡)	<0.5	<0.5	<0.5	<0.5
42	39-11(福岡)	<0.5	<0.5	<0.5	<0.5
43	39-12(福岡)	<0.5	<0.5	<0.5	<0.5
44	39-13(福岡)	<0.5	<0.5	<0.5	<0.5
45	39-14(福岡)	<0.5	<0.5	<0.5	<0.5
46	39-15(福岡)	<0.5	<0.5	<0.5	<0.5
47	40-1(佐賀)	<0.5	<0.5	<0.5	<0.5
48	40-2(佐賀)	<0.5	<0.5	<0.5	<0.5
49	42-1(熊本)	<0.5	<0.5	<0.5	<0.5
50	42-2(熊本)	<0.5	<0.5	<0.5	<0.5
51	42-3(熊本)	<0.5	<0.5	<0.5	<0.5
52	43-1(大分)	<0.5	<0.5	<0.5	<0.5
53	43-2(大分)	<0.5	<0.5	<0.5	<0.5
54	43-3(大分)	<0.5	<0.5	<0.5	<0.5
55	43-4(大分)	<0.5	<0.5	<0.5	<0.5
56	43-5(大分)	<0.5	<0.5	<0.5	<0.5
57	44-1(宮崎)	<0.5	<0.5	<0.5	<0.5
58	44-2(宮崎)	<0.5	<0.5	<0.5	<0.5
59	44-3(宮崎)	<0.5	<0.5	<0.5	<0.5
60	44-4(宮崎)	<0.5	<0.5	<0.5	<0.5
61	44-5(宮崎)	<0.5	<0.5	<0.5	<0.5
62	44-6(宮崎)	<0.5	<0.5	<0.5	<0.5
63	45-1(鹿児島)	<0.5	<0.5	<0.5	<0.5
64	45-2(鹿児島)	<0.5	<0.5	<0.5	<0.5
65	45-3(鹿児島)	<0.5	<0.5	<0.5	<0.5
66	45-4(鹿児島)	<0.5	<0.5	<0.5	<0.5
67	46-2(北海道)	<0.5	<0.5	<0.5	<0.5
68	46-3(北海道)	<0.5	<0.5	<0.5	<0.5
69	46-4(北海道)	<0.5	<0.5	<0.5	<0.5
70	46-5(北海道)	<0.5	<0.5	<0.5	<0.5
71	47-1(沖縄)	<0.5	<0.5	<0.5	<0.5
72	47-2(沖縄)	<0.5	<0.5	<0.5	<0.5
73	47-3(沖縄)	<0.5	<0.5	<0.5	<0.5
74	47-4(沖縄)	<0.5	<0.5	<0.5	<0.5
75	47-5(沖縄)	<0.5	<0.5	<0.5	<0.5
76	47-6(沖縄)	<0.5	<0.5	<0.5	<0.5
77	47-7(沖縄)	<0.5	<0.5	<0.5	<0.5
78	47-8(沖縄)	<0.5	<0.5	<0.5	<0.5
79	47-11(沖縄)	<0.5	<0.5	<0.5	<0.5
80	47-12(沖縄)	<0.5	<0.5	<0.5	<0.5

表-2 添加回収試験の結果

試験回数	結果(%)			
	AFB_1	AFB_2	AFG_1	AFG_2
1	94.1	93.6	97.4	95.4
2	98.5	99.2	99.2	95.0
3	99.0	95.3	100.1	102.7
4	95.0	93.0	100.9	95.3
5	94.5	92.2	98.0	94.5
6	97.2	95.1	103.1	96.4
7	95.3	93.7	103.0	99.6
8	92.1	91.6	96.2	93.1
平均	95.7	94.2	99.7	96.5
標準偏差(σ)	2.34	2.38	2.52	3.12

表-3 検出限界及び定量限界の算出結果

試験回数	結果(ng/g)			
	AFB_1	AFB_2	AFG_1	AFG_2
1	0.505	0.471	0.485	0.471
2	0.532	0.438	0.524	0.453
3	0.492	0.506	0.446	0.459
4	0.481	0.419	0.476	0.412
5	0.524	0.476	0.527	0.490
6	0.430	0.415	0.415	0.372
7	0.523	0.474	0.401	0.477
平均	0.498	0.457	0.468	0.447
標準偏差(σ)	0.0352	0.0337	0.0495	0.0415
3σ	0.105	0.101	0.148	0.124
10σ	0.352	0.337	0.495	0.415

5 試験方法

1) 器具及び装置

ブレンダーカップ一式(500 ml容)
シラン加工褐色試験管(10 ml容)
遠心管(50 ml容, 100 ml容)
メスフラスコ(20 ml容, 50 ml容, 100 ml容)
ガラスロート
ピペット類
メスシリンダー
リザーバー及びジョイント
ガラス繊維ろ紙(934-AH)[Whatman社]
メンブランフィルター(孔径 0.2 μm)[ジーエルサイエンス株式会社]
ワーニングブレンダー
遠心分離機
吸引マニュホールド
汎用天秤
ブロックヒーター
ボルテックスミキサー
分光光度計
高速液体クロマトグラフ(蛍光検出器付)

2) 試薬及び試液

アフラトキシンB_1, B_2, G_1及びG_2標準品[Sigma-Aldrich社]

水

メタノール(残留農薬・PCB試験用，高速液体クロマトグラフ用(以下「HPLC用」)
[和光純薬工業株式会社]

アセトニトリル(残留農薬・PCB試験用，HPLC用)[和光純薬工業株式会社]

塩化ナトリウム(試薬特級)[関東化学株式会社]

塩化カリウム(試薬特級)[和光純薬工業株式会社]

りん酸水素二ナトリウム(試薬特級)[小宗化学薬品株式会社]

りん酸二水素カリウム(試薬特級)[小宗化学薬品株式会社]

トリフルオロ酢酸(試薬特級)[Sigma-Aldrich社]

イムノアフィニティーカラム(AFLAKING)[株式会社　堀場製作所]

アセトニトリル及び水の混液(9:1)：アセトニトリル2,700 mlと水300 mlを混合した。

PBS(phosphate buffer saline)：塩化ナトリウム8 g，リン酸水素二ナトリウム1.2 g，リン酸二水素カリウム0.2 g及び塩化カリウム0.2 gを水1 Lに溶解した。

水及びアセトニトリルの混液(9:1)：水180 mlとアセトニトリル20 mlを混合した。

水，メタノール及びアセトニトリルの混液(6:3:1)：水600 ml，メタノール(HPLC用)300 ml及びアセトニトリル(HPLC用) 100 mlを混合した。

3) 試験溶液の調製

試料全量を均一に粉砕し，そのうちの50 gを正確に秤量した。これにアセトニトリル及び水の混液(9:1)200 mlを加え，ワーニングブレンダーで5分間かくはんした後，得られた抽出液を遠心分離(2,500 r/min，5分間)した。上清4 mlを正確に採取し，水で20 mlに定容したものをガラス繊維ろ紙によりろ過した。ろ液10 mlをイムノアフィニティーカラムに負荷し，PBS及び水各10 mlで順次カラムを洗浄した後，カラムを風乾した。風乾したカラムにアセトニトリル3 mlを負荷，溶出し，得られた溶出液を40 ℃以下で窒素気流下で濃縮乾固した。残さにトリフルオロ酢酸0.1 mlを加え，ボルテックスミキサーでかくはんした後，室温，暗所において15分間放置した。これに水及びアセトニトリルの混液(9:1) 0.4 mlを加えて混和後，メンブランフィルターでろ過したものを試料溶液とした。

4) 標準溶液の調製

アフラトキシンB_1, B_2, G_1及びG_2の各標準品1 mgにそれぞれアセトニトリルを加えて溶解し，100 mlに定容して10 μg/mlの標準原液を調製した。この原液を混合し，アセトニトリル及び水の混液(9:1)で適宜希釈し，アフラトキシン検量線用混合標準溶液(0.25～50 ng/ml)を調製した。

5) 検量線の作成

標準溶液20 μlを高速液体クロマトグラフに注入し，得られたアフラトキシンB_1, B_2, G_1及びG_2のピーク高さと標準溶液のアフラトキシンB_1, B_2, G_1及びG_2の濃度から検量線を作成した。

6) 高速液体クロマトグラフ法による測定

3)で得られた試験溶液20 μlを高速液体クロマトグラフに注入し，得られた目的対象物質のピーク高さと5)の検量線から，試験溶液中の目的対象物質の濃度を求め，試料中の濃度を算出した。

7) 高速液体クロマトグラフ操作条件

機　　種：LC-10ATvp[株式会社　島津製作所]
検 出 器：蛍光分光検出器 RF-10Axl[株式会社　島津製作所]
カ ラ ム：Mightysil RP-18 GP, φ4.6 mm×25 cm[関東化学株式会社]
カラム温度：40 ℃
移 動 相：水，メタノール及びアセトニトリルの混液(6:3:1)
流　　量：1.0 ml/min
測定波長：蛍光励起波長 365 nm，蛍光測定波長 450 nm
注 入 量：20 μl

8) 添加回収試験

試料中の各アフラトキシン濃度が5 ng/gになるように添加し，本分析法の操作に従って，添加回収試験(繰り返し8回)を実施して標準偏差(ばらつき)を求めた。

9) 検出限界及び定量限界の算出

試料中の各アフラトキシン濃度が0.5 ng/gになるように添加し，本分析法の操作に従って，添加回収試験(繰り返し7回)を実施して標準偏差(ばらつき)を求めた。

以　上

平成１９年度

食品・添加物等の規格基準に関する
試験検査等の実施について

食品中のトータルアフラトキシンの検査・調査

機　関　名　　財団法人　　マイコトキシン検査協会

理事長　　小　川　　博

1．目的

ピーナッツトータルアフラトキシン実態調査

本共同分析の目的は、ピーナッツのトータルアフラトキシン（B_1，B_2，G_1，G_2）の分布を調べる調査である。分析法は、アセトニトリル及び水の混液で抽出し、イムノアフィニティーカラムにより精製後、蛍光検出器を接続した高速液体クロマトグラフで検出する方法を用いる。又、輸入生落花生についてトータルアフラトキシンの汚染を調査する。

2．試験方法

1）試験対象物質

物質名	化学式	分子量
アフラトキシンB_1 (Aflatoxin B_1)	$C_{17}H_{12}O_6$	312
アフラトキシンB_2 (Aflatoxin B_2)	$C_{17}H_{14}O_6$	314
アフラトキシンG_1 (Aflatoxin G_1)	$C_{17}H_{12}O_7$	328
アフラトキシンG_2 (Aflatoxin G_2)	$C_{17}H_{14}O_7$	330

2）機器及び試薬

①イムノアフィニティーカラム（AflaKING:Horiba 製）

②アセトニトリル：高速液体クロマトグラフ用（和光純薬）

③リン酸バッファー(PBS)

④ Tween 20（和光純薬）

⑤トリフルオロ酢酸（和光純薬）

⑥アフラトキシンB_1，B_2，G_1，G_2（ACROS）

⑥高速液体クロマトグラフ（HITACHI D-7000 ｼﾘｰｽﾞ）

3）標準溶液の調製

標準品

ACROS アフラトキシン標準品B_1，B_2，G_1，G_2を各 1mg を採り AOAC 調製法により 10 μg/ml 作成し検量線用の標準溶液 1.25ng/ml~0.0625ng/ml 濃度を調製する。

4）分析法の検討

ピーナッツ実態調査に用いるアフラトキシン試験法（国立医薬品食品研究所）をローストピーナッツ及びピーナッツ菓子について添加回収試験を実施した。添加量は、アフラトキシントータル 20ppb（各 5ppb），4 ｐｐｂ（各 1ppb），2ppb（各 0.5ppb）について実施した。

5）分析操作

（イ）抽出

試料 50g をホモジナイザーカップ（500ml）に入れアセトニトリル：水(9:1) 200ml を加え５分ブレンドした後、遠心分離又はろ過する。その上清液又はろ液を 4ml とり、0.1%TweenPBS で 20ml に定容する。

（ロ）精製

イムノアフィニティーカラムの充填液を全て溶出し、イムノアフィニティーカラムに定容した 10ml を負荷し、自然落下で流出する。流出後 0.1%TweenPBS10ml で洗浄し、次に蒸留水 20ml で洗浄する。落下後カラム内の水分を全部除く。アセトニトリル 3ml で溶出する。溶出したアセトニトリルを窒素気流で濃縮乾固する。その残留物にトリフルオロ酢酸 0.1ml を加え、密栓撹拌後に暗所で１５分間放置する。水：アセトニトリル(9:1)0.4ml を加え、高速液体クロマトグラフ試験溶液とした。

（ハ）高速液体クロマトグラフ条件

機種　　：HITACHI D-7000 ｼﾘｰｽﾞ
検出器　：HITACHI D-7485 蛍光検出器
カラム　：Waters Atlantis T3 3.0 × 250mm
移動相　：水：メタノール：アセトニトリル(6:3:1)
流速　　：0.34ml/min
カラム温度：４０度
注入量　：20 μl

３．試験結果

１）検量線の作成

アフラトキシンB_1，B_2，G_1，G_2を 1.25ng/ml~0.0625ng/ml 濃度で５点作成する。

２）操作ブランク

本分析法で添加回収試料及び試料を用いず分析操作を行った結果アフラトキシンB_1，B_2，G_1，G_2のクロマトグラムの位置には夾雑物がなかった。

３）分析法の検出限界

アフラトキシンB_1，B_2，G_1，G_2の検出限界は、Ｓ／Ｎ比の３倍の 0.1ppb であった。

４）検出限界の算出

0.0625ng × 200/2 × 1000/50 ＝ 0.125 g/kg (0.125ppb)

5）添加回収試験（表－1）

ローストピーナッツ

添加量 20ppb（各 5ppb）　n＝5

	アフラトキシン B_1	アフラトキシン B_2	アフラトキシン G_1	アフラトキシン G_2
回収平均値 ppb（回収率）	4.70 (94)	4.58 (92)	4.82 (96)	4.69 (94)
標準偏差	0.28	0.25	0.37	0.29
相対標準偏差%	6.0	5.5	7.7	6.2

添加量 4ppb（各 1ppb）　n＝5

	アフラトキシン B_1	アフラトキシン B_2	アフラトキシン G_1	アフラトキシン G_2
回収平均値 ppb（回収率）	0.961 (96)	0.941 (94)	0.997 (100)	0.971 (97)
標準偏差	0.075	0.077	0.063	0.072
相対標準偏差%	7.8	8.2	6.3	7.4

添加量 2ppb（各 0.5ppb）　n＝3

	アフラトキシン B_1	アフラトキシン B_2	アフラトキシン G_1	アフラトキシン G_2
回収平均値 ppb（回収率）	0.526 (105)	0.515 (103)	0.542 (108)	0.529 (106)
標準偏差	0.075	0.05	0.08	0.068
相対標準偏差%	14.2	9.7	14.8	12.9

ピーナッツ菓子

添加量 20ppb（各 5ppb）　n＝5

	アフラトキシン B_1	アフラトキシン B_2	アフラトキシン G_1	アフラトキシン G_2
回収平均値 ppb（回収率）	5.08 (102)	4.98 (100)	5.21 (105)	4.89 (98)
標準偏差	0.15	0.21	0.24	0.2
相対標準偏差%	3.0	5.0	4.6	4.1

添加量 4ppb（各 1ppb）　n＝5

	アフラトキシン B_1	アフラトキシン B_2	アフラトキシン G_1	アフラトキシン G_2
回収平均値 ppb（回収率）	1.018 (102)	1.006 (101)	1.026 (103)	0.981 (98)
標準偏差	0.061	0.049	0.088	0.05
相対標準偏差%	6.0	4.9	8.6	5.1

6）汚染実態調査結果（表－２）

	品名	ｱﾌﾗﾄｷｼﾝ B_1	ｱﾌﾗﾄｷｼﾝ B_2	ｱﾌﾗﾄｷｼﾝ G_1	ｱﾌﾗﾄｷｼﾝ G_2
1	ﾊﾞﾀｰﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
2	ﾛｰｽﾄﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
3	みそ味ﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
4	殻付きﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
5	殻付きﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
6	ﾛｰｽﾄﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
7	ﾛｰｽﾄﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
8	ﾊﾞﾀｰﾋﾟｰﾅｯﾂ	0.2ppb	検出せず	0.2ppb	検出せず
9	ﾊﾞﾀｰﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
10	ﾛｰｽﾄﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
11	殻付ﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
12	黒糖ﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
13	ﾛｰｽﾄﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
14	ﾊﾞﾀｰﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
15	殻付ﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
16	殻付ﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
17	ﾊﾞﾀｰﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
18	ﾛｰｽﾄﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
19	ﾛｰｽﾄﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
20	殻付ﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
21	無塩落花生	検出せず	検出せず	検出せず	検出せず
22	豆菓子大粒味付ﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
23	手むき落花生	検出せず	検出せず	検出せず	検出せず
24	味付手むき落花生	検出せず	検出せず	検出せず	検出せず
25	味付ﾋﾟｰﾅｯﾂ	検出せず	検出せず	検出せず	検出せず
26	有機栽培殻付落花生	検出せず	検出せず	検出せず	検出せず
27	有機栽培殻付落花生	検出せず	検出せず	検出せず	検出せず
28	殻付き落花生	検出せず	検出せず	検出せず	検出せず
29	豆菓子落花生	検出せず	検出せず	検出せず	検出せず
30	豆菓子落花生	検出せず	検出せず	検出せず	検出せず
31	豆菓子落花生	検出せず	検出せず	検出せず	検出せず
32	豆菓子殻付落花生	検出せず	検出せず	検出せず	検出せず

検出限界 0.1ppb

３．輸入時生落花生の調査

Table3. Number of detected Aflatoxin in 2006 import of raw peanut*[1]

Country	No. Sample	Not detected No. (%)	$AFB_1 \geqq$ 10ppb No. (%)	$AFB_1 <$ 10ppb No. (%)	TotalAF*[2] $\geqq$ 20ppb No. (%)	detected AFB&G*[3] No. (%)	detected AFB*[4] No. (%)	detected only AFB1 No. (%)
Large type (vargenia,others)								
China	1654	1627 (98.4)	13 (0.8)	14 (0.8)	16 (1.0)	12 (0.7)	13 (0.8)	2 (0.1)
USA	4	4	0	0	0	0	0	0
Taiwan	4	4	0	0	0	0	0	0
Australia	1	1	0	0	0	0	0	0
Total	1663	1636 (98.4)	13 (0.8)	14 (0.8)	16 (1.0)	12 (0.7)	13 (0.8)	2 (0.1)
Small type (spanish,runner,other)								
China	576	572 (99.3)	2 (0.3)	2 (0.3)	2 (0.3)	2 (0.3)	2 (0.3)	0
S.Africa	252	247 (98.0)	2 (0.8)	3 (1.2)	3 (1.2)	3 (1.2)	1 (0.4)	1 (0.4)
USA	138	130 (94.2)	5 (3.6)	3 (2.2)	5 (3.6)	2 (1.4)	4 (2.9)	2 (1.4)
Praguay	34	32 (94.1)	0	2 (5.9)	0	0	2 (5.9)	0
Australia	13	12 (92.3)	1 (7.7)	0	1 (7.7)	1 (7.7)	0	0
India	5	4 (80.0)	1 (20.0)	0	1 (20.0)	0	1 (20.0)	0
Total	1018	997 (97.9)	11 (1.1)	10 (1.0)	12 (1.2)	8 (0.8)	10 (1.0)	3 (0.3)

Aflatoxin detection limit 0.1ppb

*[1] Result of Examination by Mycotoxin Reseach Association

*[2] Total AflatoxinB_1,B_2,G_1,G_2

*[3] Aflatoxin B_1,B_2,G_1,G_2 group

*[4] Aflatoxin B_1,B_2 group

Table 4. Ratio of Aflatoxin derivatives in 2006 import of raw peanut*[1]

Country	Ratio of Aflatoxin B&G group						Ratio of Aflatoxin B group		
	No.*[2]	AFB_1%STDEV*[3]	AFB_2%STDEV	AFG_1%STDEV	AFG_2%STDEV	AFG_1/B_1STDEV	No.*[2]	AFB_1%STDEV	AFB_2%STDEV
Large type (vargenia,others)									
China	12	16.4 ± 7.5	2.8 ± 1.9	65.7 ± 6.9	15.1 ± 5.1	4.8 ± 1.9	13	85.8 ± 6.2	14.2 ± 6.2
USA	0	—	—	—	—	—	0	—	—
Taiwan	0	—	—	—	—	—	0	—	—
Australia	0	—	—	—	—	—	0	—	—
Total		16.4 ± 7.5	2.8 ± 1.9	65.7 ± 6.9	15.1 ± 5.1	4.8 ± 1.9	13	85.8 ± 6.2	14.2 ± 6.2
Small type (spanish,runner,others)									
China	2	19.0 ± 5.0	5.8 ± 6.0	59.3 ± 16.6	16.0 ± 5.7	3.5 ± 1.8	2	87.1 ± 2.4	12.9 ± 2.4
S.Africa	3	17.7 ± 2.3	2.1 ± 0.3	67.4 ± 1.7	12.8 ± 0.9	3.8 ± 0.6	1	86.4	13.6
USA	2	33.7 ± 4.3	2.9 ± 0.4	56.2 ± 2.5	7.4 ± 2.1	1.7 ± 0.3	4	88.4 ± 3.8	11.6 ± 3.8
Praguay	0	—	—	—	—	—	2	86.4 ± 11.7	15.4 ± 11.7
Australia	1	22.5	1.8	66.7	9	3	0	—	—
India	0	—	—	—	—	—	1	86.7	13.3
Total	8	22.6 ± 7.5	3.2 ± 2.8	62.5 ± 8.3	11.8 ± 4.2	3.1 ± 1.2	10	87.0 ± 4.8	13.0 ± 4.8

*[1] Result of Examination by Mycotoxin Reseach Association

*[2] Number of detected Aflatoxin B_1,B_2,G_1,G_2 and Aflatoxin B_1,B_2

*[3] Standard deviation

４．考察

アフラトキシンは、アスペルギルス属のカビが産生し、代謝物としてＢ，Ｇグループを産生するカビとＢグループのみを産生するカビがある。日本のアフラトキシンの基準はB_1のみが対象でヨーロッパ諸国及びアメリカ等多くの国は、B_1，B_2，G_1，G_2の総計で基準[1]をもうけている。今回の報告は市販の落花生加工品及び２００６年に輸入された生落花生のアフラトキシン汚染について調査した結果である。

イムノアフィニティーカラムを使用して落花生加工品についてアフラトキシンB_1，B_2，G_1，G_2の添加回収試験を実施した。分析法の性能は、精度管理基準の回収率 70~120%以内及び相対標準偏差 15%以内の範囲内であった。（表－１）

市販品の落花生加工品の検査結果は、３２検体中１検体にアフラトキシンB_1，G_2を検出した。（表－２）

輸入時検査（表－３，４）の調査結果から大粒種（中国産）のアフラトキシンＢ，Ｇグループを検出した検数は１２検体(48%)あり、Ｂグループを検出した検数は１３検体(52%)であった。小粒種については中国産はＢ，Ｇグループを検出した検数は２検体(50%)Ｂグループを検出した検数は２検体(50%)であった。南アフリカ産はＢ，Ｇグループを検出した検数は３検体(75%)Ｂグループを検出した検数は１検体(25%)であった。アメリカ産はＢ，Ｇグループを検出した検数は２検体(33%)Ｂグループを検出した検数は４検体(67%)であった。パラグアイ産はＢグループのみで２検体であった。オーストラリア産はＢ，Ｇグループのみで１検体であった。インド産はＢグループのみで１検体であった。大粒種と小粒種の合計はＢ，Ｇグループは２０検体(46.5%)Ｂグループは２３検体(53.5%)であった。又、Ｂ，Ｇグループを検出したG_1とB_1検出量の比（表－４）は大粒種（中国）ではG_1がB_1の平均4.8 ± 1.9倍で、小粒種は平均3.1 ± 1.2倍であった。

２００６年の調査において例えば基準値を現基準のアフラトキシン$B_1$１０ｐｐｂとアメリカの基準であるトータルアフラトキシン２０ｐｐｂのダブルスタンダードに設定した場合（表－３）B_1基準で大粒種と小粒種を合わせて陽性となった２４検体(0.9%)にトータルアフラトキシン２０ｐｐｂで陽性となった大粒種３検体と小粒種１検体と合わせて２８検体(1.0%)が陽性となる。但し、Ｂグループのみ検出については、検出量がB_1がB_2より常に多いためトータルアフラトキシンになっても２０ｐｐｂ以上の陽性が増えることはなく、B_1基準の１０ｐｐｂで規制される。

５．結論

１９７２年から１９８９年[2]の小粒種の調査では、Ｂ，Ｇグループを検出したG_1とB_1の検出量の比が約１倍であったが、今回の調査結果ではＢ，Ｇグループを検出したG_1がB_1の検出量の約４倍であった。アフラトキシンG_1は発ガン性が疑われることから基準値設定はB_1だけではなくB_1，B_2，G_1，G_2も考慮すべきであることが示唆された。

但し、トータルアフラトキシンを基準値設定するには現在検査命令を実施している食品が加工品を含め多種にわたるためB_1，B_2，G_1，G_2が測定できる分析法の検討が必要である。

文献

１）Wordwide regulations for mycotoxins in food and feed in 2003

２）前田協一：マイコトキシン，31(1990).

平成１９年度

食品・添加物等規格基準に関する試験検査等の実施について

食品中のかび毒に係る汚染実態調査

機　関　名　　財団法人　日本冷凍食品検査協会

研究責任者名（契約者）　関西事業所　所長　北林　孝也

1．目的

本共同分析の目的は、ピーナッツのトータルアフラトキシンの分布を調べる調査である。分析法はアセトニトリル及び水の混液で抽出し、イムノアフィニティーカラムによる精製後、蛍光検出器を接続した高速液体クロマトグラフで検出する方法を用いる。

2．分析方法

1）分析対象化合物

アフラトキシンB1、アフラトキシンB2、

アフラトキシンG1、アフラトキシンG2

2）装置

蛍光検出器付高速液体クロマトグラフ

3）試薬、試液

①アセトニトリル

②メタノール

③蒸留水

③トリフルオロ酢酸

④イムノアフィニティーカラム（AflaKING Horiba社）

4）試験溶液の調製

粉砕試料50.0 gを正確に秤量し、アセトニトリル及び水の混液（90：10）200 mLを加えてワーニングブレンダーで5分間ブレンドする。抽出溶液はろ過したのち、ろ液上清から4.0mLをとって精製水で20.0mLとし、ガラス繊維ろ紙（Whatman934AH）でろ過する。このろ液をアフィニティーカラムに10mL負荷したのち、PBS10mL（以上）、蒸留水10mLの順で洗浄したのちカラムの中の水分を完全に出す。このカラムにアセトニトリル3mLでバイアルに溶出させ、40℃以下の窒素気流下で縮後乾固する。この残渣にトリフルオロ酢酸0.1mLを加え室温暗所で15分間放置したのち、水及びアセトニトリル（90：10）0.4mLで溶解し、孔径0.2μmのメンブランフィルターでろ過しHPLC試験溶液とする。

5）検量線の作成

各アフラトキシン標準溶液をHPLC移動相で希釈し0.1～1.0 ng/mL溶液を数点調製し、それぞれ10μLをHPLCに注入し、ピーク面積による外部標準法で検量線を作成する。

6）定量

試験溶液10μLをHPLCに注入し、5の検量線で各アフラトキシンの含量を求める。

7）測定条件

検出器：蛍光検出付高速液体クロマトグラフ

カラム：　Inertsil ODS-2、内径 4.6 mm、長さ 150m、粒径 5μm　(GL サイエンス)

カラム温度：40 ℃

流速：1.0mL/min

検出波長：Ex 365nm, Em 450nm

移動相：アセトニトリル：メタノール：水（1：3：6）

8）定量限界、検出限界

定量限界　0.1 ng/g

検出限界　0.04ng/g

分析成績表

		トータルアフラトキシン ng/g			
	サンプル番号	B1	B2	G1	G2
1	7-1	–	–	–	–
2	7-2	–	–	–	–
3	7-3	–	–	–	–
4	7-4	–	–	–	–
5	7-5	–	–	–	–
6	7-6	–	–	–	–
7	7-7	–	–	–	–
8	7-8	–	–	–	–
9	7-9	–	–	–	–
10	7-10	–	–	–	–
11	7-11	–	–	–	–
12	7-12	–	–	–	–
13	7-13	–	–	–	–
14	7-14	–	–	–	–
15	12-2	–	–	–	–
16	12-3	–	–	–	–
17	13-1	–	–	–	–
18	13-2	–	–	–	–
19	13-3	–	–	–	–
20	13-4	–	–	–	–
21	13-5	–	–	–	–
22	13-6	–	–	–	–
23	13-7	–	–	–	–
24	13-8	–	–	–	–
25	13-9	–	–	–	–
26	13-10	–	–	–	–
27	13-11	–	–	–	–
28	13-12	–	–	–	–
29	13-13	–	–	–	–
30	13-14	–	–	–	–
31	15-2	–	–	–	–
32	19-1	–	–	–	–
33	19-2	–	–	–	–
34	19-3	–	–	–	–
35	20-1	–	–	–	–
36	20-2	–	–	–	–
37	21-1	–	–	–	–
38	21-2	–	–	–	–
39	21-3	–	–	–	–
40	22-1	–	–	–	–

分析成績表

		トータルアフラトキシン ng/g			
	サンプル番号	B1	B2	G1	G2
41	22-2	−	−	−	−
42	22-3	−	−	−	−
43	22-4	−	−	−	−
44	22-5	−	−	−	−
45	22-6	−	−	−	−
46	22-7	−	−	−	−
47	22-8	−	−	−	−
48	22-9	−	−	−	−
49	22-10	−	−	−	−
50	22-11	−	−	−	−
51	22-12	−	−	−	−
52	22-13	−	−	−	−
53	22-14	−	−	−	−
54	22-15	−	−	−	−
55	22-21	−	−	−	−
56	22-22	−	−	−	−
57	22-23	−	−	−	−
58	22-24	−	−	−	−
59	23-1	−	−	−	−
60	23-2	−	−	−	−
61	23-3	−	−	−	−
62	24-1	−	−	−	−
63	24-2	−	−	−	−
64	24-3	−	−	−	−
65	24-4	−	−	−	−
66	24-5	−	−	−	−
67	24-6	−	−	−	−
68	24-7	−	−	−	−
69	24-8	−	−	−	−
70	24-9	−	−	−	−
71	24-10	−	−	−	−
72	25-1	−	−	−	−
73	26-1	−	−	−	−
74	26-2	−	−	−	−
75	26-3	−	−	−	−
76	26-4	−	−	−	−
77	26-5	−	−	−	−
78	27-1	−	−	−	−
79	27-2	−	−	−	−
80	27-3	−	−	−	−

（－）検出限界以下(＜0.04ng/g)

変更点

LCカラム：ODS, 150×4.6mm,5μ
注入量： 10μL

	B1	B2	G1	G2
回収率 (0.5ppb)	105.9	105.1	96.1	97.9
回収率 (5ppb)	110.9	104.8	112.0	103.2
LOD (ng/g)	0.04	0.04	0.04	0.04
LOQ (ng/g)	0.1	0.1	0.1	0.1

2. European Food Safety Authority:Opinion of the scitific panel CONTAM related to the potential increase of consumer health risk by a possible increase of the exicting maximum levels for aflatoxins in almonds, hazelnuts and pistachios and derived productspublication Date , 1 March 2007) 全文 (英文)

European Food Safety Authority

Aflatoxins in food

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Aflatoxins in food: EFSA assesses new proposed maximum levels for almonds, hazelnuts and pistachios and advises the European Commission

Last updated: 2 March 2007
Publication Date: 2 March 2007

EFSA has assessed, at the request of the European Commission (EC), the possibility of a potential increase in consumers' health risks if higher levels of aflatoxins would be permitted for almonds, hazelnuts and pistachios. In its opinion EFSA's Panel dealing with contaminants in the food chain (CONTAM Panel) concluded that increasing the maximum levels of aflatoxins in these three nuts would have only minor effects on the expected total dietary exposure from all sources and the risk of cancer. However, EFSA's scientific experts pointed out that it is essential to keep aflatoxin exposure from food sources as low as reasonably achievable by reducing exposure from the sources that are major contributors to total dietary exposure to aflatoxins.

Aflatoxins occur naturally in foods such as nuts, figs and other dried fruits, spices and crude vegetable oils. They are produced by moulds that grow on plants before harvest or on the foods during storage. They are undesirable because they have been shown to cause cancer in animals and humans.

The EC asked for this opinion in the context of discussions at meetings of the Codex Alimentarius Commission of the FAO/WHO (Food and Agricultural Organization of the United Nations and the World Health Organization). Whilst the EU maximum levels for processed almonds, hazelnuts and pistachios are currently 4 µg/kg total aflatoxins[1], the Codex Alimentarius Commission proposed in 2005 to set levels for total aflatoxins of 15 µg/kg for unprocessed almonds, hazelnuts and pistachios. At its 2006 meeting, levels of 8 µg/kg for these three ready-to-eat nuts were discussed, but no final decision has been taken.

These proposed maximum levels of aflatoxins for the three nuts, to be set at an international level, are aimed to facilitate worldwide trade. The EC represents the European Union at the Codex Alimentarius Commission meetings. EFSA's opinion provides risk managers with the scientific basis for responding to these proposals.

The Panel felt that keeping aflatoxin exposure from food sources as low as reasonably achievable was important to protect public health. The experts emphasised the importance of reducing the number of highly contaminated foods reaching the market, as well as reducing exposure from other foods, not just these nuts. The Panel concluded that increasing the maximum levels of aflatoxins for almonds, pistachios and hazelnuts would have only a minor effect on the estimated total dietary exposure of people from all sources and therefore on cancer risk. In its assessment the Panel also took into account high level consumers.

Estimated dietary exposures for children were within the range of estimates for the adult population. The main contributors to aflatoxin intakes in children were from foods other than nuts for which data specific to children's diets were not available.

The opinion on aflatoxins in foods is published at
http:/www.efsa.europa.eu/en/science/contam/contam_opinions/ej446_aflatoxins.html

Codex Alimentarius Commission
The Codex Alimentarius Commission was created in 1963 by FAO and WHO to develop food standards, guidelines and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme. The main purposes of this Programme are to protect the health of consumers, ensure fair trade practices and promote coordination of all food standards work undertaken by international governmental and non-governmental organizations. More information about the work of the Codex Alimentarius Commission can be found at
http:/www.codexalimentarius.net/web/index_en.jsp.
The next Codex Committee on Contaminants in Foods will take place from 16 to 20 April 2007 in Beijing (China).

[1] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (**http:/eur-lex.europa.eu/LexUriServ/site/en/oj/2006/l_364/l_36420061220en00050024.pdf**)

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European Food Safety Authority

CONTAM Opinions

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Opinion of the Scientific Panel CONTAM related to the potential increase of consumer health risk by a possible increase of the existing maximum levels for aflatoxins in almonds, hazelnuts and pistachios and derived products

Last updated: 1 March 2007
Publication Date: 1 March 2007

Adopted on 25 January 2007. (Question Nº EFSA-Q-2006-174)

- Opinion
- Summary

Summary

Aflatoxins are produced by moulds that are especially found in areas with hot, humid climates. They are most likely to contaminate tree nuts, ground nuts, figs and other dried fruits, spices, crude vegetable oils, cocoa beans and maize. Because aflatoxins are considered to be genotoxic and carcinogenic, it is not possible to identify an intake without risk, and the European Union (EU) introduced regulations for these toxins in 1998, at levels considered to be as low as reasonably achievable. Recent discussions in Codex Alimentarius have proposed setting worldwide a maximum level for total aflatoxins in unprocessed almonds, hazelnuts and pistachios, higher than that currently in force in the EU. As a result, the Scientific Panel on Contaminants in the Food chain (CONTAM) was asked to advise on the potential increase in risks to consumer health associated with a proposed change of the currently existing EU maximum level of 4 µg/kg for total aflatoxins (sum of aflatoxins B1, B2, G1 and G2) in almonds, hazelnuts and pistachios to 8 or 10 µg/kg, taking into account consumption patterns of these nuts in the EU, and intake of aflatoxins from other foods.

About 40,000 analytical results on occurrence of aflatoxins in various food commodities were considered by the CONTAM Panel. Aflatoxins were not detected in about 75% of the samples tested, i.e. if present they were below the limit of detection of the methods used, which varied for different sets of data. For those samples where aflatoxins were detectable, aflatoxin B1 was generally the major contributor to total aflatoxins. As a conservative estimate for the purposes of the evaluation, the CONTAM Panel assumed that total aflatoxins would be a maximum of twice the level of aflatoxin B1. The CONTAM Panel also received data relating to concentrations of aflatoxin M1 (the major metabolite of aflatoxin B1) in commercial milk samples. For almost all of these data, the values for the aflatoxin M1 concentration were below 0.05 µg/kg and taking into account the lower carcinogenic potency of M1 the Panel did not consider these data further.

In order to assess the impact of a possible change in the maximum levels for almonds, hazelnuts and pistachios, the CONTAM Panel estimated dietary exposure excluding occurrence data above 4, 8 and 10 µg/kg, respectively. These calculations indicated that increasing the maximum levels of total aflatoxins from 4 to 8 or 10 µg/kg could result in slight absolute increases in total aflatoxins in almonds, hazelnuts and pistachios, but that the mean concentrations would remain below 1 µg/kg according to the submitted test results.

Assessment of the impact of these potential increases in aflatoxin occurrence requires consideration of both the consumption of these three types of nut and the intake from other dietary sources. Robust data on other sources of dietary exposure, representative of all Member States, were not available to the CONTAM Panel. Evaluation of the few available national dietary exposure data indicated that a reasonable approximation of European diets could be obtained from the GEMS/Food Consumption Cluster Diets database, and the CONTAM Panel therefore used these data in estimating dietary exposure to aflatoxins from foods other than almonds, hazelnuts and pistachios. The contribution from these nuts was only a few percent of total dietary exposure to aflatoxins.

The estimates indicated that increasing the maximum levels for total aflatoxins in almonds, hazelnuts and pistachios from 4 to 8 or 10 µg/kg would result in an increase in average total dietary exposure to aflatoxins in the region of 1%.

Data on consumption of almonds, hazelnuts and pistachios were available from few Member States, and all were subject to limitations. The CONTAM Panel therefore assessed the range of possible exposure data for high level consumers ranging from the Member State with the lowest consumption to that with the highest consumption of each type of nut. These estimates indicated that increasing the maximum levels for total aflatoxins from 4 to 8 or 10 µg/kg could increase total dietary exposure to aflatoxins by up to 20% in consumers with the highest level of consumption. If, as is expected, nuts

exceeding the maximum levels are occasionally consumed, the total long term average dietary exposures might be higher, but the relative impact of raising the maximum level from 4 to 8 or 10 µg/kg in the three nuts would be less.

Estimated dietary exposures for children were within the range of estimates for adult populations, however these were predominated by exposure from foods other than nuts, for which data specific to children' s diets were not available.

The available data on pre-export controls on hazelnuts and pistachios indicated that increasing the maximum level for total aflatoxins from 4 to 8 or 10 µg/kg might allow up to 6% extra batches of nuts onto the EU market. No pre-export data were available for almonds.

A number of epidemiological studies have shown clear associations between aflatoxin exposure and incidence of hepatocellular carcinoma in areas with high prevalence of chronic hepatitis B, which is itself a risk factor for liver cancer. The CONTAM Panel noted considerable uncertainties in the cancer potency estimates, particularly with respect to the relevance of the data generated in the most sensitive strain of rodents and in the human data regarding the predominance of a single study of a population with high prevalence of chronic hepatitis B and very high aflatoxin exposure estimates. This study showed much greater liver cancer risk than in other populations studied. The CONTAM Panel could not discount any of these data and therefore compared the estimated dietary exposures with all of these assessments.

Assessments of cancer risks based on potency estimates derived by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and applied to the estimated average and high level dietary exposures to aflatoxins were at least two orders of magnitude lower than the reported incidences of hepatocellular carcinoma in Europe, suggesting that aflatoxins are unlikely to be a major contributor to hepatocellular carcinoma in the EU.

The margins of exposure (MOEs) calculated by the CONTAM Panel for all estimated intakes compared with the 95% lower confidence limit of the benchmark dose for a 10% increase in cancer incidence (BMDL10) based on animal data indicated a potential concern for human health. BMDL10 and BMDL1[1] values derived from data from human populations including the most sensitive subgroups with high prevalence of chronic hepatitis B infection, indicated similar sensitivity of this population to that of the most sensitive strain of rat, but that other subgroups are likely to be less sensitive to the effects of aflatoxins.

The CONTAM Panel concluded that changing the maximum levels for total aflatoxins from 4 to 8 or 10 µg/kg in almonds, hazelnuts and pistachios would have minor effects on the estimates of dietary exposure, cancer risk and the calculated MOEs.

The CONTAM Panel concluded that exposure to aflatoxins from all sources should be as low as reasonably achievable, because aflatoxins are genotoxic and carcinogenic. The data indicate that the reduction of total dietary exposure to aflatoxins could be achieved by reducing the number of highly contaminated foods reaching the market and reducing exposure from food sources other than almonds, hazelnuts and pistachios.

[1] 95% lower confidence limit of the benchmark dose for a 1 % increase in cancer incidence

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OPINION OF THE SCIENTIFIC PANEL ON CONTAMINANTS IN THE FOOD CHAIN ON A REQUEST FROM THE COMMISSION RELATED TO THE POTENTIAL INCREASE OF CONSUMER HEALTH RISK BY A POSSIBLE INCREASE OF THE EXISTING MAXIMUM LEVELS FOR AFLATOXINS IN ALMONDS, HAZELNUTS AND PISTACHIOS AND DERIVED PRODUCTS

Question N° EFSA-Q-2006-174

Adopted on 25 January 2007

SUMMARY

Aflatoxins are produced by moulds that are especially found in areas with hot, humid climates. They are most likely to contaminate tree nuts, ground nuts, figs and other dried fruits, spices, crude vegetable oils, cocoa beans and maize. Because aflatoxins are considered to be genotoxic and carcinogenic, it is not possible to identify an intake without risk, and the European Union (EU) introduced regulations for these toxins in 1998, at levels considered to be as low as reasonably achievable. Recent discussions in Codex Alimentarius have proposed setting worldwide a maximum level for total aflatoxins in unprocessed almonds, hazelnuts and pistachios, higher than that currently in force in the EU. As a result, the Scientific Panel on Contaminants in the Food chain (CONTAM) was asked to advise on the potential increase in risks to consumer health associated with a proposed change of the currently existing EU maximum level of 4 µg/kg for total aflatoxins (sum of aflatoxins B1, B2, G1 and G2) in almonds, hazelnuts and pistachios to 8 or 10 µg/kg, taking into account consumption patterns of these nuts in the EU, and intake of aflatoxins from other foods.

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Estimated dietary exposures for children were within the range of estimates for adult populations, however these were predominated by exposure from foods other than nuts, for which data specific to children's diets were not available.

The available data on pre-export controls on hazelnuts and pistachios indicated that increasing the maximum level for total aflatoxins from 4 to 8 or 10 µg/kg might allow up to 6% extra batches of nuts onto the EU market. No pre-export data were available for almonds.

A number of epidemiological studies have shown clear associations between aflatoxin exposure and incidence of hepatocellular carcinoma in areas with high prevalence of chronic hepatitis B, which is itself a risk factor for liver cancer. The CONTAM Panel noted considerable uncertainties in the cancer potency estimates, particularly with respect to the relevance of the data generated in the most sensitive strain of rodents and in the human data regarding the predominance of a single study of a population with high prevalence of chronic hepatitis B and very high aflatoxin exposure estimates. This study showed much greater liver cancer risk than in other populations studied. The CONTAM Panel could not discount any of these data and therefore compared the estimated dietary exposures with all of these assessments.

Assessments of cancer risks based on potency estimates derived by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and applied to the estimated average and high level dietary exposures to aflatoxins were at least two orders of magnitude lower than the reported incidences of hepatocellular carcinoma in Europe, suggesting that aflatoxins are unlikely to be a major contributor to hepatocellular carcinoma in the EU.

The margins of exposure (MOEs) calculated by the CONTAM Panel for all estimated intakes compared with the 95% lower confidence limit of the benchmark dose for a 10% increase in cancer incidence (BMDL10) based on animal data indicated a potential concern for human health. BMDL10 and BMDL1[1] values derived from data from human populations including the most sensitive subgroups with high prevalence of chronic hepatitis B infection, indicated similar sensitivity of this population to that of the most sensitive strain of rat, but that other subgroups are likely to be less sensitive to the effects of aflatoxins.

The CONTAM Panel concluded that changing the maximum levels for total aflatoxins from 4 to 8 or 10 µg/kg in almonds, hazelnuts and pistachios would have minor effects on the estimates of dietary exposure, cancer risk and the calculated MOEs.

The CONTAM Panel concluded that exposure to aflatoxins from all sources should be as low as reasonably achievable, because aflatoxins are genotoxic and carcinogenic. The data indicate that the reduction of total dietary exposure to aflatoxins could be achieved by reducing the number of highly contaminated foods reaching the market and reducing exposure from food sources other than almonds, hazelnuts and pistachios.

KEY WORDS

Aflatoxins, aflatoxin B1, almonds, hazelnuts, pistachios, exposure assessment, hepatocellular carcinoma, liver cancer, risk assessment, margin of exposure (MOE).

[1] 95% lower confidence limit of the benchmark dose for a 1 % increase in cancer incidence

The EFSA Journal (2007) 446, 1 - 127

OPINION OF THE SCIENTIFIC PANEL ON CONTAMINANTS IN THE FOOD CHAIN ON A REQUEST FROM THE COMMISSION RELATED TO THE POTENTIAL INCREASE OF CONSUMER HEALTH RISK BY A POSSIBLE INCREASE OF THE EXISTING MAXIMUM LEVELS FOR AFLATOXINS IN ALMONDS, HAZELNUTS AND PISTACHIOS AND DERIVED PRODUCTS

Question N° EFSA-Q-2006-174

Adopted on 25 January 2007

SUMMARY

Aflatoxins are produced by moulds that are especially found in areas with hot, humid climates. They are most likely to contaminate tree nuts, ground nuts, figs and other dried fruits, spices, crude vegetable oils, cocoa beans and maize. Because aflatoxins are considered to be genotoxic and carcinogenic, it is not possible to identify an intake without risk, and the European Union (EU) introduced regulations for these toxins in 1998, at levels considered to be as low as reasonably achievable. Recent discussions in Codex Alimentarius have proposed setting worldwide a maximum level for total aflatoxins in unprocessed almonds, hazelnuts and pistachios, higher than that currently in force in the EU. As a result, the Scientific Panel on Contaminants in the Food chain (CONTAM) was asked to advise on the potential increase in risks to consumer health associated with a proposed change of the currently existing EU maximum level of 4 µg/kg for total aflatoxins (sum of aflatoxins B1, B2, G1 and G2) in almonds, hazelnuts and pistachios to 8 or 10 µg/kg, taking into account consumption patterns of these nuts in the EU, and intake of aflatoxins from other foods.

About 40,000 analytical results on occurrence of aflatoxins in various food commodities were considered by the CONTAM Panel. Aflatoxins were not detected in about 75% of the samples tested, i.e. if present they were below the limit of detection of the methods used, which varied for different sets of data. For those samples where aflatoxins were detectable, aflatoxin B1 was generally the major contributor to total aflatoxins. As a conservative estimate for the purposes of the evaluation, the CONTAM Panel assumed that total aflatoxins would be a maximum of twice the level of aflatoxin B1. The CONTAM Panel also received data relating to concentrations of aflatoxin M1 (the major metabolite of aflatoxin B1) in commercial milk samples. For almost all of these data, the values for the aflatoxin M1 concentration were below 0.05 µg/kg and taking into account the lower carcinogenic potency of M1 the Panel did not consider these data further.

In order to assess the impact of a possible change in the maximum levels for almonds, hazelnuts and pistachios, the CONTAM Panel estimated dietary exposure excluding occurrence data above 4, 8 and 10 µg/kg, respectively. These calculations indicated that

increasing the maximum levels of total aflatoxins from 4 to 8 or 10 µg/kg could result in slight absolute increases in total aflatoxins in almonds, hazelnuts and pistachios, but that the mean concentrations would remain below 1 µg/kg according to the submitted test results.

Assessment of the impact of these potential increases in aflatoxin occurrence requires consideration of both the consumption of these three types of nut and the intake from other dietary sources. Robust data on other sources of dietary exposure, representative of all Member States, were not available to the CONTAM Panel. Evaluation of the few available national dietary exposure data indicated that a reasonable approximation of European diets could be obtained from the GEMS/Food Consumption Cluster Diets database, and the CONTAM Panel therefore used these data in estimating dietary exposure to aflatoxins from foods other than almonds, hazelnuts and pistachios. The contribution from these nuts was only a few percent of total dietary exposure to aflatoxins.

The estimates indicated that increasing the maximum levels for total aflatoxins in almonds, hazelnuts and pistachios from 4 to 8 or 10 µg/kg would result in an increase in average total dietary exposure to aflatoxins in the region of 1%.

Data on consumption of almonds, hazelnuts and pistachios were available from few Member States, and all were subject to limitations. The CONTAM Panel therefore assessed the range of possible exposure data for high level consumers ranging from the Member State with the lowest consumption to that with the highest consumption of each type of nut. These estimates indicated that increasing the maximum levels for total aflatoxins from 4 to 8 or 10 µg/kg could increase total dietary exposure to aflatoxins by up to 20% in consumers with the highest level of consumption. If, as is expected, nuts exceeding the maximum levels are occasionally consumed, the total long term average dietary exposures might be higher, but the relative impact of raising the maximum level from 4 to 8 or 10 µg/kg in the three nuts would be less.

Estimated dietary exposures for children were within the range of estimates for adult populations, however these were predominated by exposure from foods other than nuts, for which data specific to children's diets were not available.

The available data on pre-export controls on hazelnuts and pistachios indicated that increasing the maximum level for total aflatoxins from 4 to 8 or 10 µg/kg might allow up to 6% extra batches of nuts onto the EU market. No pre-export data were available for almonds.

A number of epidemiological studies have shown clear associations between aflatoxin exposure and incidence of hepatocellular carcinoma in areas with high prevalence of chronic hepatitis B, which is itself a risk factor for liver cancer. The CONTAM Panel noted considerable uncertainties in the cancer potency estimates, particularly with respect

to the relevance of the data generated in the most sensitive strain of rodents and in the human data regarding the predominance of a single study of a population with high prevalence of chronic hepatitis B and very high aflatoxin exposure estimates. This study showed much greater liver cancer risk than in other populations studied. The CONTAM Panel could not discount any of these data and therefore compared the estimated dietary exposures with all of these assessments.

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KEY WORDS

Aflatoxins, aflatoxin B1, almonds, hazelnuts, pistachios, exposure assessment, hepatocellular carcinoma, liver cancer, risk assessment, margin of exposure (MOE).

[1] 95% lower confidence limit of the benchmark dose for a 1 % increase in cancer incidence

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LIST OF ABBREVIATIONS AND ACRONYMS

AACC	American Association of Cereal Chemistry
AF-alb	Aflatoxin-albumin
AFAR	Aflatoxin aldehyde reductase
AFB1	Aflatoxin B1
AFB1-N7-Gua	8,9-dihydro-8-(N7-guanyl)-9-hydroxy-AFB1
AFB1-FAPY	AFB1-formamidopyrimidine
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AFM1	Aflatoxin M1
AFP1	Aflatoxin P1
AFQ1	Aflatoxin Q1
AFSSA	Agence Française de Securité Sanitaire des Aliments
ALARA	As low as reasonably achievable
AOAC	Association of Official Analytical Chemists
BfR	Bundesinstitut für Risikobewertung
BGYF	Bright greenish-yellow fluorescence
BMD	Benchmark dose
BMDL	BMD lower limit
CCFAC	Codex Committee on Food Additives and Contaminants
CONTAM	Scientific Panel on Contaminants in the Food chain of EFSA
CYPs	Cytochrome P-450 enzymes
EC	European Commission
EFSA	European Food Safety Authority
EFTA	European Free Trade Association
ELISA	Enzyme-linked immunosorbent assay
EPIC	European Prospective Investigation into Cancer and Nutrition
FAO	Food and Agriculture Organisation
FFQ	Food frequency questionnaire
FRUCOM	European Federation of the Trade in Dried Fruit, Edible Nuts, Processed Fruit & Vegetables, Processed Fishery Products, Spices, Honey and Similar Foodstuffs
GSH	Glutathione
GST	Glutathione-S-transferases
$HBsAg^+$	Hepatitis B virus antigen positive
$HBsAg^-$	Hepatitis B virus antigen negative
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HPLC	High pressure liquid chromatography
IARC	International Agency for Research on Cancer
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee for Food Additives
LB	Lower bound
LC-MS	Liquid chromatography-mass spectrometry
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
LOQ	Limit of quantification
ML	Maximum level
MOE	Margin of exposure
NER	Nucleotide excision repair
NOAEL	No observed adverse effect level

PCD	Post-column derivatisation
SC	Scientific Committee of EFSA
SCF	Scientific Committee for Food
SCOOP	EU Scientific Co-operation Assessment Project
TD_{50}	Tumorigenic dose rate 50
TLC	Thin layer chromotography
TOF	Time-of-flight
UB	Upper bound
WHO	World Health Organisation

BACKGROUND

Toxicology of aflatoxins

The Scientific Committee for Food (SCF) concluded in 1994 that "aflatoxins are genotoxic carcinogens. For this type of carcinogen, it is generally felt that there is no threshold dose below which no tumour formation would occur. In other words, only a zero level of exposure will result in no risk. From many reports on risk assessment, it can be concluded that even very low levels of exposure to aflatoxins, i.e. 1 ng/kg b.w. per day still contribute to the risk of liver cancer".

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) performed in 1997 a quantitative risk assessment by estimation of the population risks from intake of aflatoxins, comparing two hypothetical standards (10 µg/kg and 20 µg/kg). Acknowledging the several limitations and assumptions inherent in this approach, the JECFA concluded in case of a low prevalence of hepatitis B, that reducing the hypothetical standard from 20 µg/kg to 10 µg/kg yields a drop in the estimated population risk of approx. 2 cancers/year per 10^9 people (and in case of a high prevalence of hepatitis B: 300 cancers/year per 10^9 people).

The SCF considered this assessment in September 1997 and concluded that it was not possible to assess the degree of uncertainty, arising from these limitations and assumptions, in the quantitative risk assessment and felt therefore that it was premature for SCF to draw definitive conclusions on this issue. The toxicology of the aflatoxins was not questioned by the JECFA which concluded "aflatoxins are amongst the most potent mutagenic and carcinogenic substances known" and therefore the SCF concluded that its opinion of 1994 remained valid.

EU measures on aflatoxins

The presence of contaminants is always undesirable in food, but in many cases unavoidable. For aflatoxins, the EU applied the ALARA principle in 1998 by adopting strict maximum levels at concentrations as low as reasonably achievable.
Harmonised maximum levels for aflatoxins have been in place in the EU since 1 January 1999 and are laid down in the Annex, Section 2 of Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs[2]. For groundnuts, nuts, dried fruit, cereals and processed products thereof intended for direct human consumption or as an ingredient in foodstuffs, maximum levels of 4 µg/kg for total aflatoxins (aflatoxins B1 +B2 + G1 + G2) and 2 µg/kg for aflatoxin B1 (AFB1) have been fixed. For spices corresponding levels have been set to 10 µg/kg for total aflatoxins and 5 µg/kg for AFB1.

[2] OJ L 364, 20.12.2006, p.5-24.

Sorting techniques and other possible physical treatments which reduce the aflatoxin content can be carried out on unprocessed groundnuts, nuts, dried fruit and maize to obtain the final consumer product. Taking these techniques into account, higher maximum levels for groundnuts (15 µg/kg for total aflatoxins and 8 µg/kg for AFB1), nuts, maize and dried fruit (10 µg/kg for total aflatoxins and 5 µg/kg for AFB1) to be subjected to a sorting or other physical treatment, before their human consumption or their use as an ingredient in foodstuffs are proposed. In the case of raw products, to which the higher limit applies, the destination of these products and precise intended use must be clearly demonstrated by labelling. It is also prohibited to detoxify products by chemical treatments.

For milk and milk products, a maximum level of 0.05 µg/kg for aflatoxin M1 has been established.

Recognising the need to ensure consumer protection from exposure to aflatoxins, the Commission safeguard decision 2006/504/EC has been adopted requiring stricter controls on certain products originating from certain countries. Monitoring data has demonstrated that the products below, mostly nuts, require further safety reassurance from producing countries as well as up to 100% sampling and analysis at import:

- Brazil nuts in shell from Brazil,
- peanuts from China,
- peanuts from Egypt,
- pistachios from Iran,
- figs, hazelnuts and pistachios from Turkey.

Codex Alimentarius

Discussions in Codex in 2005

A level of 15 µg/kg for total aflatoxins has been discussed in 2005 in Codex Committee on Food Additives and Contaminants (CCFAC) for unprocessed and processed almonds, hazelnuts and pistachios.

The EC has indicated during that meeting in 2005 to be eventually in a position to accept 15 µg/kg for total aflatoxins for unprocessed almonds, hazelnuts and pistachios, but that a level of 15 µg/kg for total aflatoxins for processed almonds, hazelnuts and pistachios was not acceptable. The large majority of the other Codex Member Countries were in favour of the level of 15 µg/kg for total aflatoxins for processed and unprocessed almond, hazelnuts and pistachios. No consensus could be reached at last year's meeting and the discussion was postponed to this year's meeting in April 2006.

The CCFAC decided to circulate for comments at Step 3 the proposed draft maximum level of total aflatoxins of 15 µg/kg in processed almonds, hazelnuts, and pistachios (ALINORM 05/28/12, § 141 and Appendix XXII)

Discussions in Codex in 2006

During the meeting of CCFAC this year the EC has taken the following position:

Position of the EC as regards the maximum level for total aflatoxin in processed almonds , hazelnuts and pistachios

Taking into account the comments made by other Member Countries in CCFAC on this issue, the EC is in favour of finding a common solution which meets to a large extent the concerns and comments made by the different member Countries.

Therefore, the EC will not oppose the forwarding of ***a level of 8 µg/kg total aflatoxins in processed almonds, hazelnuts and pistachios*** *for* ***adoption at Step 5*** *by the Codex Alimentarius Commission.*

However, concern has been expressed as regards the possible public health consequences given that this level signifies a significant increase compared to the current EU maximum level of 4 µg/kg. Therefore the EC will request its scientific body, the European Food Safety Authority (EFSA), to provide a risk assessment in order to clarify if acceptance of this higher level would not entail unacceptable risks for the EU consumer, taking into account vulnerable groups in the population and also the significant increase in tree nut consumption in recent years in the EU.

Therefore, it is clearly stated that a ***possible final acceptance*** *of that level at Step 8 will* ***depend on the outcome of this risk assessment*** *which is expected to be available prior to the next session of the Codex Committee dealing with Contaminants in Food.*

In addition, the EC is of the opinion that it is necessary that ***producing countries shall be required*** *to provide the Codex Committee dealing with Contaminants in Food, by its next session, with* ***detailed information as regards the implementation*** *of the "Code of Practice for the prevention and reduction of aflatoxin contamination in tree nuts" adopted by the Codex Alimentarius Commission at its 28th session in 2005 (CAC/RCP 59-2005).*

Position of the EC as regards the maximum level for total aflatoxins in unprocessed almonds, hazelnuts and pistachios

The 28th Session of the Codex Alimentarius Commission adopted the proposed maximum level for total aflatoxins in unprocessed almonds, hazelnuts, and pistachios at Step 5 and advanced it to Step 6. (ALINORM 05/28/41, § 71 and Appendix VIII)

The European Community indicated that a possible future acceptance of this level for unprocessed almonds hazelnuts and pistachios at Step 8 of the Codex uniform procedure will depend on the outcome of the ongoing discussions on the maximum level for total aflatoxins in processed almonds, hazelnuts, and pistachios (ALINORM 05/28/41, § 76)

Therefore the EC is of the position that ***the draft maximum level of 15 µg/kg for total aflatoxins in unprocessed almonds, hazelnuts and pistachios should be retained at step*** *6 until the next Session of Codex Committee dealing with Contaminants in Food."*

Outcome of the discussions in Codex

The Codex Committee on Food Additives and Contaminants (CCFAC) decided at its meeting in April 2006 to establish an electronic Working Group, led by the European Community, to elaborate a discussion paper on the aflatoxin level in ready-to-eat tree nuts, considering

i) the detailed data on distribution of aflatoxins between lots,
ii) consumer health risk assessment of different levels of aflatoxins in ready-to-eat tree nuts,
iii) sampling plan for aflatoxin contamination in almonds, Brazil nuts, hazelnuts and pistachios,
iv) effects of codes of practice, and
v) terminology of "ready-to-eat" and "for further processing" for circulation, comments and consideration at the next session.

The CCFAC also agreed to request JECFA to conduct a dietary exposure assessment on tree nuts (ready-to-eat), in particular, almonds, hazelnuts, pistachios, and Brazil nuts, as well as to assess the impact on exposure taking into account hypothetical levels of 4, 8, 10 and 15 µg/kg, put in the context of exposure from other sources and previous exposure assessments on maize and groundnuts

The CCFAC felt it important to show progress while awaiting further data on the impact of alternate levels and agreed to advance to Step 5 the proposed maximum level of 8 µg/kg for total aflatoxins in ready-to-eat almonds, hazelnuts and pistachios. The delegation of Iran expressed its reservation to this decision (ALINORM 06/29/12 - §§ 131 and 132 and Appendix XXII).

The European Community (EC) accepted the adoption of the level of 8 µg/kg for total aflatoxins in ready-to-eat almonds, hazelnuts and pistachios at Step 5 by the Codex Alimentarius Commission at its 29th Session in July 2006.

However, the EC clearly indicated that a possible future acceptance of this level for ready-to-eat almonds hazelnuts and pistachios at Step 8 of the Codex uniform procedure would depend on the consideration of the different issues which will be addressed in the discussion paper on the aflatoxin level in ready-to-eat nuts (ALINORM 06/29/12, § 129) in particular

- the consumer health risk assessment of different levels of aflatoxins in ready-to-eat nuts
- the sampling plan for aflatoxin contamination in almonds, Brazil nuts, hazelnuts and pistachios
- the effects of codes of practice.

Request for a scientific opinion from EFSA

Assessment of potential increase of consumer health risk

EFSA is requested to provide an opinion on the potential increase of consumer health risk by a possible increase of the maximum levels from 4 to 8 and 10 µg/kg for total aflatoxins in "ready-to-eat" almonds, hazelnuts and pistachios and derived products. Current European maximum levels are 2 µg/kg for AFB1 and 4 µg/kg for total aflatoxins. The current proposal to increase limits for total aflatoxins does not include any provisions to set limits for AFB1.

In this assessment Member States' specific consumption patterns should be considered given that the consumption of tree nuts is very diverse in the EU and particular attention should also be paid to specific (vulnerable) groups of the population, including children and high level consumers. In this assessment, EFSA is requested to take into account the exposure to all aflatoxins, including aflatoxin M1, from other food sources.

The Commission will provide occurrence data on the presence of AFB1 and total aflatoxins in a wide range of food items obtained in the time period of 2000 – 2006 and provide data available on Member States' specific consumption data on nuts and nut products, in particular almonds, hazelnuts and pistachios.

Analytical results reported by the laboratory are given as the best estimate values, corrected for recovery, but do not take into account the measurement uncertainty. Exposure assessments do not take into account measurement uncertainty as it may lead to underestimates of exposure. However, when comparing analytical results to maximum levels, this is done on the best estimate that is corrected for recovery minus measurement uncertainty. It is appropriate to consider this when assessing the potential increase of consumer health risk as the consequence of an increase of maximum levels (e.g. the measurement of uncertainty for total aflatoxins in the range of 4 to 10 µg/kg can be around 40%).

In considering these data, EFSA is requested to take into account, insofar as is toxicologically relevant, the relative proportion of AFB1 to total aflatoxins. Furthermore, it should also consider that the provided occurrence data have been obtained in a period where the current existing maximum levels were in force and that a change in the pattern

of distribution of aflatoxins present in food could occur if higher maximum levels were in force.

National risk assessments have been already performed by Germany and France and it is requested that EFSA considers these national assessments, as well as other national assessments which might become available shortly.

Also the JECFA will conduct a dietary exposure assessment on tree nuts (ready-to-eat), in particular, almonds, hazelnuts, and pistachios, Brazil nuts, and impact on exposure taking into account hypothetical levels of 4, 8, 10 and 15 µg/kg, put in the context of exposure from all other sources and previous pertinent exposure assessments

Margin of exposure (MOE)

The JECFA decided at its 64th meeting in 2005 that advice on compounds that are both genotoxic and carcinogenic should be based on estimated Margins of Exposure (MOEs). The strengths and weaknesses inherent in the data used to calculate the MOE should be given as part of the advice to risk managers, together with advice on its interpretation. At its 64th meeting, the JECFA estimated MOEs for acrylamide, ethylcarbamate and PAHs.

Also the Scientific Committee (SC) of the European Food Safety Authority recommends the application of the MOE approach as a harmonised methodology for assessing the risk of genotoxic and carcinogenic substances which may be found in food and feed, irrespective of their origin.

The EFSA is requested to estimate the MOE for the presence of aflatoxins (total aflatoxins /AFB1) in food, taking into particular consideration more vulnerable groups of the population, such as children and high level consumers (for example vegetarians) and carriers of hepatitis. For comparative reasons, it would be appropriate for the MOE to be (also) calculated on a comparable basis to the already estimated MOEs by international experts groups such as the JECFA.

TERMS OF REFERENCE

In accordance with Art. 29 (1) of Regulation (EC) No 178/2002 the European Commission asks the European Food Safety Authority to provide a scientific opinion

- on the potential increase of consumer health risk by a possible increase of the currently existing maximum level of 4 µg/kg to 8 and 10 µg/kg for total aflatoxins in "ready-to-eat" almonds, hazelnuts and pistachios and derived products taking into account the exposure to aflatoxins (B1, B2, G1, G2, M1) from other food sources considering
  - occurrence data provided and the uncertainties related to the heterogeneous distribution of aflatoxins;
  - specific consumption patterns of the relevant food commodities in the different Member States;
  - specific (vulnerable) groups of the population, including children, hepatitis carriers and high level consumers;
  - relative proportion of AFB1 to total aflatoxins.

- on the margin of exposure (MOE) for the presence of aflatoxins (total aflatoxins /AFB1) in food, considering different vulnerable groups of the population, including children and high level consumers.

ASSESSMENT

1. Introduction

1.1 General information

Aflatoxins are difuranocoumarins produced primarily by two species of *Aspergillus* fungus which are especially found in areas with hot, humid climates. *A. flavus* is ubiquitous, favouring the aerial parts of plants (leaves, flowers) and produces B aflatoxins. *A. parasiticus* produces both B and G aflatoxins, is more adapted to a soil environment and has more limited distribution. The structures of the key aflatoxins are shown in figure 1.

Aflatoxins are found in food as a result of fungal contamination both pre- and post-harvest, with the rate and degree of contamination dependent on temperature, humidity, soil and storage conditions. They are most commonly associated with groundnuts, tree nuts, dried fruit, spices, figs, crude vegetable oils, cocoa beans, maize, rice, cottonseed and copra. Aflatoxin M1 is a major metabolite of aflatoxin B1 (AFB1) in humans and animals and may be present in milk from animals fed on AFB1 contaminated feed. Exposure to aflatoxins is generally considered to occur mainly from imported materials. It is currently uncertain whether future changes in climate in the EU would lead to increased aflatoxin contaminants.

Unless otherwise stated in the opinion total aflatoxins refer to the sum of aflatoxins B1, B2, G1, and G2.

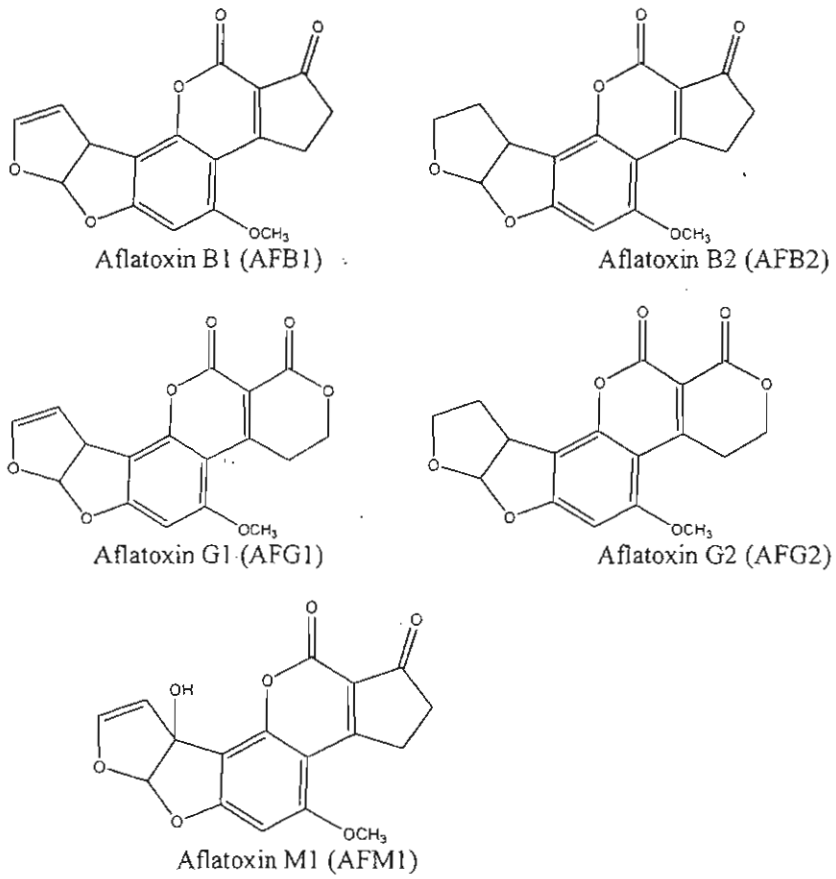

Figure 1: Structures of the B and G aflatoxins and of aflatoxin M1.

The health effects of aflatoxins have been reviewed by a number of expert groups. The International Agency for Research on Cancer (IARC) has concluded that naturally occurring aflatoxins are carcinogenic to humans (group 1), with a role in aetiology of liver cancer, notably among subjects who are carriers of hepatitis B virus (HBV) surface antigens. In experimental animals there was sufficient evidence for carcinogenicity of naturally occurring mixtures of aflatoxins and of aflatoxins B1, G1 and M1, limited evidence for aflatoxin B2 and inadequate evidence for aflatoxin G2. The principal tumours were in the liver, although tumours were also found at other sites including the kidney and colon. AFB1 is consistently genotoxic *in vitro* and *in vivo* (IARC, 1993 and 2002).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that aflatoxins are amongst the most potent mutagenic and carcinogenic substances known (FAO/WHO, 1998). The JECFA estimated potency values for AFB1 from the

epidemiological data. More details are given in chapter 4.1.8. These corresponded to 0.3 cancers/year per 100,000 population per ng aflatoxin/kg b.w. per day (uncertainty range: 0.05-0.5) in hepatitis B virus antigen positive individuals and 0.01 cancers/year per 100,000 population per ng aflatoxin/kg b.w. per day (uncertainty range: 0.002-0.03) in hepatitis B virus antigen negative individuals.

The Scientific Committee for Food (SCF) endorsed the IARC conclusions in 1994 and concluded that even very low levels of exposure to aflatoxins could contribute to risk of liver cancer (EC, 1996). The SCF reconfirmed its opinion in 1997 (EC, 1997a). The CONTAM Panel previously evaluated AFB1 as an undesirable substance in animal feed (EFSA, 2004). The Panel noted that there is emerging evidence of potential for aflatoxin contamination of feed materials grown in areas of Southern Europe where a subtropical climate and extensive agricultural practice favour fungal growth and subsequent formation of aflatoxins.

Aflatoxin M1 has been evaluated separately from AFB1 by the JECFA, because of its potential to be present in milk and milk products of livestock fed on aflatoxin-contaminated feed (FAO/WHO, 2001). The JECFA concluded that aflatoxin M1 should be presumed to induce liver cancer in rodents by a similar mechanism to AFB1, and that estimates of the potency of AFB1 can be used for determining the risk due to intake of aflatoxin M1, including those for populations with a high prevalence of carriers of hepatitis B virus. The carcinogenic potency of aflatoxin M1 was estimated to be one-tenth that of AFB1, based on a comparative study in the Fischer rat conducted by Cullen *et al.* (1987).

The CONTAM Panel has been asked to advise on risks to consumer health associated with a possible increase in maximum levels (MLs) of aflatoxins in almonds, hazelnuts and pistachios. The JECFA analysed the application of two hypothetical standards to the risk of liver cancer in model populations (FAO/WHO, 1998). It concluded that when a substantial fraction of the food supply is heavily contaminated, reducing the aflatoxin contamination levels may lower liver cancer rates. However, when only a small fraction of the food supply is heavily contaminated, reducing the standard by an apparently substantial amount may have little appreciable effect on public health.
The Panel was provided with two national risk assessments addressing the terms of reference of the current evaluation. These opinions have taken different approaches to the estimation of the potency of aflatoxins and also to the exposure assessment.

The Bundesinstitut für Risikobewertung (BfR) noted that the benchmark dose for a 10% increase in tumour incidence (BMD10) in laboratory animals was 150 ng/kg b.w. per day for AFB1 (information provided to EFSA, BfR 2006[3]). The BfR did not describe the data

[3] This is cited as a BMD10 in the BfR document as a result of an error in documents prepared for a conference on risk assessment of substances that are both genotoxic and carcinogenic (Barlow *et al.*, 2006) but in fact corresponds to the BMDL10 cited in O'Brien *et al.* (2006)

or models used to derive this value. It was assumed that other aflatoxins were equally potent, as a worst case scenario. The Agence Française de Securité Sanitaire des Aliments (AFSSA) referred to the estimates of cancer risk derived by the JECFA, for populations with and without hepatitis B antigen, and estimated a prevalence of 1% hepatitis B antigen carriers in the European population (information provided to EFSA; AFSSA, 2006).

The BfR estimated exposure from the 95th percentile consumption by children aged from two to under five years, of all foods containing aflatoxins at the current permitted MLs. An aflatoxin intake level of 25 ng/kg b.w. per day was calculated and regarded as a reasonable worst case value. For average consumption, the aflatoxin intake was estimated to be 3 ng/kg b.w. per day. Hazelnuts and almonds were major contributors to children's intakes, and increasing the MLs for hazelnuts, almonds and pistachios from 4 to 10, 15, or 20 µg/kg was estimated to increase the theoretical average intake from 3 to 3.8, 4.4 or 5 ng/kg b.w. per day, respectively.

The AFSSA estimated exposure using three different scenarios, the first being similar to that of the BfR assuming all foods contain aflatoxins at the current MLs. At the current MLs, exposure estimates for adults were 12 and 20 ng/kg b.w. per day and for children aged 3 to 14 years were 23 and 43 ng/kg b.w. per day at the average and 95th percentile, respectively. In these exposure estimates consumption of hazelnuts, almonds and pistachios was very low, and increasing the MLs had no impact on the total exposure estimates.

The BfR estimated Margins of Exposure (MOEs) by dividing the BMDL10 of 150 ng/kg b.w. per day by exposure estimates at the current MLs. The MOEs were 50 and 6 for average and high level consumer aged two to under five, respectively. Increasing the MLs for hazelnuts, almonds and pistachios to 10, 15, or 20 µg/kg would reduce the MOE for average consumers of this age to 39, 34 and 30, respectively.

The AFSSA used the adult exposure data to predict the number of increased cancer cases in the French population. If all foods contained aflatoxins at the current maximum permitted levels, the estimated average exposure of 12 ng/kg b.w. per day would result in estimated increases of 1.5 cases/10^6 people per year.

Hence the major differences between the BfR and the AFSSA evaluations are in the data for consumption of hazelnuts and almonds, and in allowing for the decreased sensitivity of the 99% of the French population considered to be hepatitis B virus antigen negative.

1.2 Sampling techniques

Aflatoxins may be very heterogeneously distributed within a lot, in particular in a lot with a large particle size, such as dried figs or groundnuts. Therefore, sampling as well as

analysis plays a crucial role in the accuracy and precision of the determination of aflatoxins in food commodities. As a consequence, harmonized methods of sampling and analysis for the official control of the levels of aflatoxins and other mycotoxins in foodstuffs are laid down in Commission Regulation (EC) No. 401/2006 of 23 February 2006[4]. This Regulation repealed Commission Directives 98/53/EC, 2002/26/EC, 2003/78/EC and 2005/38/EC which until then laid down the sampling methods and the methods of analysis for the official control of the levels for aflatoxins, ochratoxin A, patulin and Fusarium toxins in foodstuffs. The new Regulation was issued in order to embrace the relevant requirements for a representative sampling and reliable analysis for all mycotoxins in a single legal act.

Besides definitions and general provisions, Annex I of Regulation (EC) No. 401/2006[4] stipulates detailed requirements for methods of sampling for different food products. The requirements differ depending on particle size, lot weight and the form in which the commodities are placed on the market in order to obtain a comparable representativeness. Regarding the particle size of the food products, the provisions for the method of sampling for the official control of aflatoxin compliance with the MLs laid down in Regulation (EC) No. 1881/2006[2] distinguishes between

- Cereals and cereal products;
- Dried fruit, including dried vine fruit and derived products with the exception of dried figs;
- Dried figs, groundnuts and nuts;
- Spices and
- Milk and milk products, infant formulae and follow-on formulae, including infant milk and follow-on milk.

Each lot, which is to be examined, shall be sampled separately. In accordance with the specific sampling provisions for the respective mycotoxins, large lots shall be subdivided into sublots to be sampled separately. In general, for batches with food products with large particle size, the weight of the aggregate sample as the combined total of all incremental samples taken from the lot or sublot is larger than in case of batches with food products with a smaller particle size. For example, depending on lot weight the aggregate sample of dried figs, groundnuts and nuts can weigh up to 30 kg. In case the groundnuts and nuts are not subjected to further sorting or physical treatment, the aggregate sample shall be mixed and divided into three equal laboratory samples which have to be analysed separately. Since the distribution of aflatoxins and other mycotoxins in processed products is generally considered less heterogeneous than in the unprocessed products, simpler sampling provisions are laid down for processed products.

Sampling of foodstuffs at the retail stage must be done where possible in accordance with the provisions set out in Annex I of Regulation (EC) No. 401/2006[4]. Where that is not

[4] OJ L70, 9.3.2006, p.12-34.

possible, an alternative method of sampling at retail stage may be applied provided that it ensures that the aggregate sample is sufficiently representative of the sampled lot and is fully described and documented. In any case, the aggregate sample shall be at least 1 kg. In case the portion to be sampled is so small that it is impossible to obtain an aggregate sample of 1 kg, the aggregate sample weight of foodstuffs sampled at the retail stage might be less than 1 kg.

Additional practical information for competent authorities being in charge for the control of compliance with EU legislation on aflatoxins is given in a guidance document which is subordinate to the provisions of Regulation (EC) No. 401/2006[4].

1.3 Analytical methods

Since the early 1960s different types of analytical techniques have been applied for the identification of aflatoxin-contaminated agricultural commodities. An early non-chemical screening test is the "bright greenish-yellow fluorescence (BGYF)" or "black light" test. Suspected samples e.g. corn or figs are inspected under a UV-lamp. The characteristic fluorescence under long-wave ultraviolet light (365 nm) is associated with the presence of kojic acid formed by aflatoxin producing fungi like *A. flavus* or *A. parasiticus* (Ashworth and McMeans, 1966). The BGYF test indicates the growth of the fungi that may have resulted in the production of aflatoxins.

Thin layer chromatography (TLC) became the first technique capable of detecting and quantifying aflatoxins in food at low levels (Nesheim *et al.*, 1964). Before TLC analysis, the aflatoxins are extracted from the sample, usually with an aqueous organic solvent, and the extract is purified by one or more techniques such as solvent partitioning, column filtration or chromatography. After spotting sample and standard solutions onto the plates these are developed using a suitable solvent. The aflatoxins on the developed TLC-plates are identified and estimated under long-wave UV light either visually or by densitometry measuring the intensity of fluorescence of the aflatoxin spots. Due to their strong bluish (AFB1+AFB2) and greenish (AFG1+AFG2) fluorescence under long wave ultraviolet light approximately 0.5 ng per spot can be routinely detected either visually or by densitometry.

In 1968 small chromatographic columns (minicolumns) were introduced for the detection of aflatoxins in food and food extracts (Holaday, 1968). In principle, the minicolumns are used in a manner similar to the TLC-method. The minicolumns consist of a glass tubing containing the packing material e.g. silica gel or a combination of different packing materials. After dipping into the sample extract the minicolumn is placed in a beaker containing a "developing solvent" which is drawn up the column by capillary action. After 10 to 15 minutes the column is examined under long wave ultraviolet light for the characteristic blue or bluish - green colour that the aflatoxins emit when exited by light of this wavelength. The limit of detection ranges between 5 and 20 µg/kg. The main

advantage of commercially available minicolumns is that they are easy to use and give results within 25 minutes. They are mainly used as "go or no go" field tests to accept or reject a truckload or railway car of peanuts or corn. Some kinds of these methods have also become official methods of the Association of Official Analytical Chemists (AOAC), the American Association of Cereal Chemistry (AACC), and the International Union of Pure and Applied Chemistry (IUPAC).

Since the mid 1970s, immuno-chemical methods based on highly specific antibodies against aflatoxins have been developed. Until now many types of enzyme-linked immunosorbent assays (ELISA) are commercially available. These assays include ELISA-techniques for the analysis of a huge number of samples with (semi)-quantitative results as well as rapid and easy to use "go or no go" field tests. All enzyme-linked immunosorbent assays have to follow a strict protocol depending on the kind of sample and the solvents used. Otherwise cross-reacting matrix compounds as well as unsuited solvents can influence the binding of the aflatoxins to the antibody as well as affect the following enzymatic reaction leading to false negative as well as to false positive results.

All techniques described so far can be used as "go or no go" field tests as well as for laboratory screening tests to reduce the time necessary to test samples that do not contain a detectable amount of aflatoxins.

In the EU, methods of analysis for the official control (enforcement, defence and referee purposes) of the levels of aflatoxins and other mycotoxins in foodstuffs have to fulfil the analytical requirements laid down in Annex II of Commission Regulation (EC) No 401/2006 of 23 February 2006[4]. These include *inter alia* criteria for laboratory blanks, recovery and precision and specify that the analytical result corrected for recovery shall be used for controlling compliance.

The most successful analytical method used for aflatoxins in food which meets the required performance criteria laid down in this Regulation is based on immuno-affinity column clean up followed by High Performance Liquid Chromatography (HPLC) with post-column derivatisation (PCD) and fluorescence detection (Stroka *et al.*, 2000). Before fluorescence detection the aflatoxins have to undergo a derivatisation reaction. The derivatisation is necessary because the fluorescence of AFB1 and AFG1 undergoes quenching under the aqueous conditions in RP-chromatography. This quenching is suppressed after reaction of the AFB1 and AFG2 with iodine or bromine. In most applications PCD is performed either by addition of pyridinium bromide perbromide to the eluate or by reaction with electrochemically generated bromine. The latter is achieved by addition of potassium bromide to the mobile phase, which releases bromine in a special electrochemical reactor. Due to the highly selective clean-up and concentration of the aflatoxins by immuno-affinity chromatography in combination with post-column derivatisation and fluorescence detection, limits of detection down to 0.01 µg/kg can be achieved applying this method.

Until now, many analytical methods for determination of aflatoxins, either for screening or for confirmation purposes have been successfully developed. However, results gained with methods based on TLC, immuno-chemical assays as well as HPLC-techniques with UV or fluorescence detection are usually not considered as proof of identity of an analyte, especially if legal consequences are involved. For this reason, the combination of liquid chromatography with mass spectrometry (LC-MS) will become the method of choice because it provides direct information on the chemical structure of an analyte. Therefore, more and more laboratories use LC-MS techniques for the confirmation of aflatoxins and other mycotoxin contamination. The improvement and availability of different types of mass spectrometers, such as quadrupole, ion-trap, time-of-flight instruments (TOF) and combinations thereof allows not only the confirmation of mycotoxins but will also lead to powerful multi-residue methods for mycotoxin analysis in the near future (Sulyok *et al.*, 2006).

2. Legislation on aflatoxins

Harmonised MLs for aflatoxins have been in place in the EU since 1 January 1999 and are laid down in the Annex, Section 2 of Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting MLs for certain contaminants in foodstuffs[5]. For groundnuts, nuts, dried fruit, several species of spices and cereals, including buckwheat and processed products thereof intended for direct human consumption or as an ingredient in foodstuffs, MLs for AFB1 as well as the sum of aflatoxins B1 + B2 + G1 + G2 have been fixed.

Sorting techniques and other possible physical treatments, which reduce the aflatoxin content can be carried out on unprocessed groundnuts, nuts, dried fruit and maize to obtain the final consumer product. Taking these techniques into account, higher MLs for groundnuts, nuts, maize and dried fruit to be subjected to a sorting or other physical treatment, before their human consumption or their use as an ingredient in foodstuffs are established (see also background). MLs are also set for AFB1 in baby foods and processed cereal-based foods for infants and young children as well as for dietary foods for special medical purposes intended specifically for infants.

As AFB1 is metabolized into AFM1 in ruminants that have consumed contaminated feed, MLs were established for AFM1 in milk and milk products as well as infant formulae and follow-on formulae including infant milk and follow-on milk.

Under Article 5 of Regulation (EEC) No 315/93[6], Member States may maintain their national provisions concerning the MLs for aflatoxins in certain foodstuffs for which no Community provisions have been adopted. As a consequence, a number of national

[5] OJ L 364, 20.12.2006, p.5-31.

[6] OJ L 37, 12.2.1993, p.204-206.

regulations for specific food commodities in various Member States still exist beside the harmonized EU MLs.

An international inquiry performed by the Dutch National Institute for Public Health and the Environment in 2003 describes the situation of worldwide mycotoxin regulation (FAO, 2004). On a worldwide basis, at least 99 countries had mycotoxin regulations or guidelines for food and/or feed in 2003 in force. The aflatoxin regulations are often detailed and specific for various foodstuffs, for dairy products and for feedstuffs. Regarding AFB1, the worldwide accepted levels in food range between 1 and 20 µg/kg. In 2003, a ML of 2 µg/kg was in force in 29 countries. Most of these countries belonged to the 15 EU Member States and accession countries as well as to the European Free Trade Association (EFTA). A ML of 5 µg/kg was set in 21 countries spread over Africa, Asia/Oceania, Latin America and Europe. Some countries, such as the United States and Canada do not have a separate ML for AFB1.

Concerning the sum of aflatoxins B1, B2, G1 and G2, the worldwide accepted levels range between 0 and 35 µg/kg. The harmonized EU ML of 4 µg/kg is applied by 29 countries again mainly EU and EFTA countries. A ML of 20 µg/kg for the sum of aflatoxins B1, B2, G1 and G2 was harmonized by MERCOSUR (Mercado Común del Sur, Southern Common Market) a customs union between Argentina, Brazil, Uruguay, Paraguay and Venezuela and is meanwhile applied in a total of 17 countries, with half of them in Latin America. Also the United States follows this 20 µg/kg ML.

3. Assessment of human exposure

Though a wide range of foods may be contaminated with aflatoxins, they have been most commonly associated with tree nuts, groundnuts, figs and other dried fruits, spices, crude vegetable oils, cocoa beans, maize, rice, cottonseed and copra. At the forty-ninth meeting of the JECFA an attempt was made to quantify exposure to aflatoxins in the diet (FAO/WHO, 1998). Data from Australia indicated an average estimated intake of 0.15 ng/kg b.w. per day of aflatoxins with the upper 95th percentile diet containing approximately twice that level. A series of Chinese intake and market basket studies reported intakes ranging from 0 to 91 µg/kg b.w. per day of AFB1. The USA reported an eaters-only mean lifetime intake of total aflatoxins of 0.3 ng/kg b.w. per day and intake for the 90th percentile individuals at 0.7 ng/kg per day. The EU in the Scientific Co-operation Assessment Project (SCOOP) on aflatoxins indicated an intake range of 0.03 to 1.3 ng/kg b.w. per day for AFB1.

The JECFA report noted that many assumptions in the intake estimates were made that biased them upwards. The JECFA concluded that quantitative estimates of intake of aflatoxins at the international level are severely limited by the lack of representative data. Although intake estimates are available at the national level for many countries, the submitters of all of these studies were emphatic in stating that the results are not truly

"representative". In general, the results appear to be biased upwards because monitoring studies focus on lots of commodities that are thought to be contaminated. It is thus important to clarify the impact of uncertainty and variability in data for aflatoxin exposure assessments.

3.1 Methodology of exposure assessment

Uncertainty and variability in exposure estimates

The dietary exposure assessment component of a risk assessment combines food consumption data with data on the concentration of chemicals in food. The resulting dietary exposure estimate is then compared with the relevant toxicological profile of the food chemical of concern. In calculating the exposure estimate it is important to have a clear understanding of uncertainties and variability associated with data used in establishing occurrence levels of the chemical and consumption patterns of relevant foods.

Uncertainty is a measure of the precision in the methodology used to quantify the parameter. Variability is a measure of the inherent heterogeneity of the material studied. They are both important to take into account when arriving at and considering the final exposure estimate.

For the current opinion, occurrence data on aflatoxins in a range of food products were collected from random and targeted monitoring and surveillance activities in Member States over a seven year period and from some other sources. Laboratories in Member States used different analytical methods, as described previously in chapter 1.3, with different sensitivities. Some reported only results from the use of immuno-affinity screening columns, while others used high performing HPLC/fluorescence detection methods with a limit of detection 1,000 times or more lower. Since it cannot be assumed that samples with aflatoxins below the limit of detection are free of contamination, the limit of detection becomes very important in estimating actual levels. The uncertainty associated with the limit of detection can be reduced by using more sensitive methods, however, less sensitive methods are still feasible for food surveillance purposes. There is thus a dichotomy between scientific and surveillance needs that cannot be easily resolved. Subsequently, a range of different levels of detection was reported for submitted test results used in this opinion contributing to a considerable amount of the uncertainty associated with the final estimate.

Uncertainty is also introduced by the food selection method for product to be tested. Targeted monitoring will introduce a sampling bias and increase uncertainty of the estimate for general use. This is particularly important because of the variability in the distribution of aflatoxins in any food product. Selective sampling of anticipated problem commodities can result in aflatoxin levels many times higher than a representative sample. The heterogeneity in the aflatoxin distribution across a food lot is a well-known

issue caused by localised mould growth and was also reflected in results from multiple sampling of the same lot presented in this opinion. Random monitoring is the better method for exposure assessment to reduce uncertainty. Most results submitted to the Panel came from the official food control in respective Member States and would comprise both random and targeted sampling thus introducing both uncertainty and variability. The exact split between random and targeted sampling is not known.

Food consumption data can be collected at different levels of precision. Several different methods were used in the current opinion. The GEMS/Food consumption cluster diets database (FAO/WHO, 2006) consists of food balance data and can only be used as a proxy for food consumption associated with a large amount of uncertainty. Data from the more accurate food diary method were also reported from some Member States and used in some of the comparisons. Irrespective of the methods, use of food consumption recordings are coupled with large uncertainties and a huge variability between individuals within the overall population, especially in case of single food items. Extrapolation to specific population subgroups, like children, vegetarians and hepatitis B carriers in this case, creates further difficulties in data interpretation.

The main question to be addressed by the Panel was a comparison of the effects on aflatoxin exposure of varying the permitted MLs in almonds, hazelnuts and pistachios taking into account the exposure from other foods. An actual assessment of such a question can only be undertaken if the efficiency of prevention and enforcement activities is known and an accurate background level of aflatoxin exposure can be established. Relative occurrence of aflatoxins from pre-export, import and market testing were determined. However, the food categories used varied, there were seasonal variations and sampling and testing methodologies were different.

Although a range of different food products were tested for the presence of aflatoxins, it proved very difficult to identify clear-cut levels for estimation of total dietary exposure to aflatoxins. The Panel therefore had to use a number of assumptions and approximations in these estimates and it is recommended that further research be undertaken to remove some of the uncertainty associated with the estimate.

A deterministic model has been used for the calculation of the exposure assessment estimates in this report. To account for some of the described uncertainty and variability, different scenarios were developed and tested covering a range of possible values. Rather than one exposure estimate, the result is presented as a plausible range using the best available science.

3.2 Occurrence data

Data description

A total of 49,748 analytical results on occurrence of aflatoxins were submitted from 22 EU Member States in response to a call for information issued by the European Commission. The close to 5,700 data from the Netherlands could not be used because only aggregated data were submitted and the nearly 700 data from Lithuania were incomplete and only showed approval or rejection of imported lots. Due to incomplete product description, approximately 4,700 data from two Italian regions could also not be used. Overall 38,648 sample results from Member States were entered into the database. On close analysis a further 4,332 sample results had to be discarded because of deficiencies in the way results were presented or the limit of detection of the method used was not adequate for the analysis (see limit of detection below). In total, 34,326 analytical results submitted by Austria (1,453), Belgium (434), Cyprus (212), Czech Rep (1,464), Denmark (340), Estonia (349), Finland (1,419), France (2,719), Germany (5,287), Greece (4,847), Hungary (3,750), Ireland (1,765), Italy (6,959), Latvia (549), Luxembourg (320), Slovakia (939), Slovenia (402), Spain (229), Sweden (211) and United Kingdom (678) were included in the following analysis. Submissions were also received from Turkey and FRUCOM (European Federation of the Trade in Dried Fruit, Edible Nuts, Processed Fruit & Vegetables, Processed Fishery Products, Spices, Honey and Similar Foodstuffs). Turkey reported individual analytical results from testing of 6,762 hazelnut and pistachio samples from the official pre-export control that were analysed in 2005 and 2006. FRUCOM reported internal food business compliance testing results from 2002 to 2006 covering approximately 3,500 samples consisting of mainly aggregated data with an indicated non conformity rate of about 1%. Because of the data aggregation, the FRUCOM results could not be incorporated into further analysis. However, the Turkish data will be presented in some detail as a separate part of the analysis (see section 3.3).

AFM1 test results from analyses of commercial milk samples were received in separate submissions and data extracted from the SCOOP report (EC, 1997b). The SCOOP report contained 10,871 analytical results reported by Member States from testing in 1989-1995 with just 34 samples (0.3%) above the ML of 0.05 µg/kg. In 4,993 results reported by Estonia, Finland, Germany and the United Kingdom from testing in 2000-2006 only five samples (0.1%) showed values above the limit of detection of which only one (0.02%) exceeded the ML of 0.05 µg/kg. However, in an Italian submission of 789 results from testing of milk and cheese in 2002 to 2006 about 6% showed values above 0.05 µg/kg with almost half of the positive results coming from testing of cheese in 2004.

In the call for information, Member States were asked to indicate what type of control the respective samples related to (import, market or company control) and if the product was market ready or would undergo further processing before being sold, since the latter would allow some further sorting and thus reduction of aflatoxin levels. The country of origin of the product was also requested. All such requested information was not forthcoming for all samples tested and analyses of such factors have thus been performed

on a sub-sample of the overall material, but only those for which a sufficient amount of data was available.

The information covers seven years from 2000 to 2006 (Table 1). Information for 2006 was incomplete as the deadline for submission was the end of September 2006.

Results were grouped into 14 food categories as shown in Table 1 with special emphasis on almonds, hazelnuts and pistachios.

Table 1: Distribution of samples over year and food category.

Food category	Number of samples						
	2000	2001	2002	2003	2004	2005	2006
All data	2883	3609	4386	5605	8313	6638	2890
Almonds	112	108	206	362	347	287	344
Baby foods	0	0	42	282	113	134	21
Brazil nuts	28	181	142	130	61	71	9
Cashews	7	23	21	51	77	107	50
Figs	85	145	301	444	571	431	90
Hazelnuts	100	170	569	673	739	642	270
Maize	70	66	55	306	258	122	66
Other cereals	417	479	207	240	539	618	510
Other dried fruits	107	75	179	242	283	347	163
Other foodstuffs	159	138	135	250	444	345	133
Other nuts	88	104	119	204	233	274	109
Peanuts	1260	1600	1451	1057	1640	1366	555
Pistachios	246	384	428	680	1062	917	352
Spices	204	136	531	684	1947	977	219

Reporting of aflatoxin concentrations

Aflatoxin concentrations were reported as below the limit of detection (LOD) for 25,451 of the European samples while aflatoxins above the LOD were found in 8,875 or 26% of samples. For the samples where aflatoxins were not detected it has to be assumed that concentrations ranged between zero and the LOD. As recommended by the FAO/WHO (1995) for materials where the majority of results are below the limit of detection, both lower and upper bound values were calculated to provide an estimate of a concentration range. Thus, the respective LOD was entered as the actual value (upper bound) or replaced by zero (lower bound). The impact of the two methods is illustrated in Figure 2 for total aflatoxins in the European samples. There is a maximum difference of 0.46 µg/kg at the 80th percentile after which the difference levels out as the levels reach the LOD. The lower bound mean is 5% lower than the upper bound mean, or 4.28 and 4.53 µg/kg, respectively.

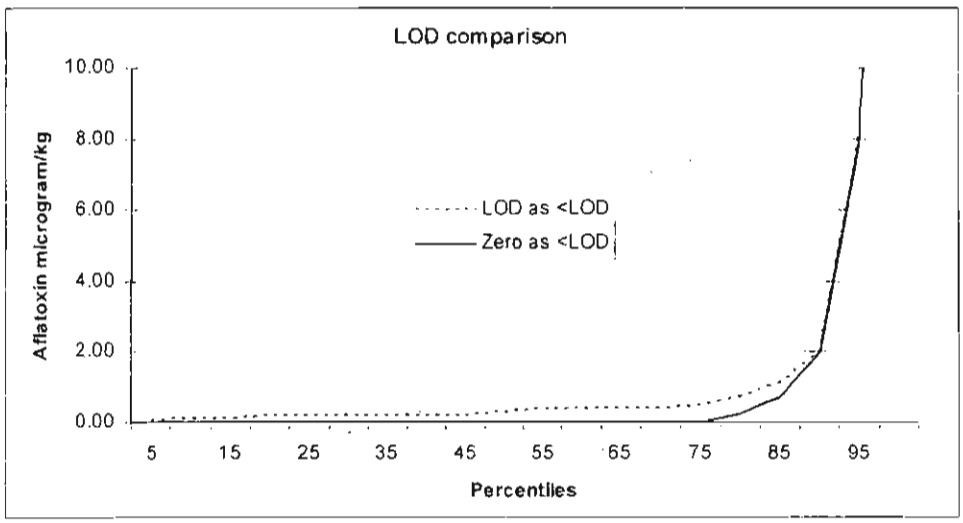

Figure 2: Comparison of the impact of using the LOD or zero for all total aflatoxin values <LOD.

The LOD for the aflatoxins varied considerably between laboratories, different foods, methods used and over time in that more sensitive methods were adopted. The minimum LOD reported for AFB1 was 0.0002 µg/kg and the maximum 10 µg/kg, but usually the LOD was reported at around 0.1 µg/kg. Some laboratories reported only the limit of quantification (LOQ). LOD is most often equal to three times the standard deviation of a blank or a low concentration sample while LOQ is ten times the standard deviation or 3.3 times the LOD. Results reported as less than the LOQ were thus transformed to LOD using this relationship and assuming there were no detected concentrations of aflatoxins. Furthermore, samples with a LOD above 1 µg/kg for AFB1 (equivalent to 2 µg/kg of for total aflatoxins as shown below) were excluded from the analysis as recommended by the FAO/WHO (1995) since the sensitivity was too close to or above the level of interest for the study. Thus, around 4,000 results from mainly the use of screening methods had to be discarded.

The distribution of the LOD for all samples remaining in the analysis is shown in Figure 3. The most common LOD was 0.1 or 0.2 µg/kg for AFB1 and 0.2 or 0.4 µg/kg for total aflatoxins after the adjustment indicated below.

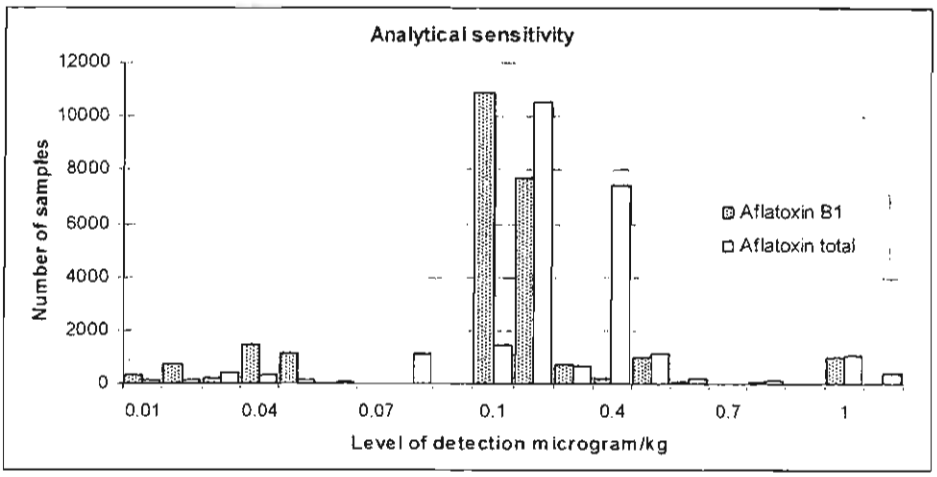

Figure 3: The limit of detection as applied by laboratories for all samples in the study.

The LOD for total aflatoxins as reported by Member States was comprised of the sum of the separate LODs for aflatoxins B1, B2, G1 and G2 and was often four times the level for AFB1 or more. In the experience of the Panel, AFB1 most often dominated the mix of aflatoxins in food samples and it was expected that simply adding together the LODs for all aflatoxins would considerably overstate the total aflatoxin level. To check this assumption, the relationship between AFB1 and total aflatoxins was calculated through linear regression for values above the limit of detection (Table 2).

Table 2: Calculation of the relationship between concentrations of AFB1 and total aflatoxins in the different food categories utilising all samples above the LOD.

Food category	No of samples All	No of samples >LOD	Linear regression coefficient[1]	R^2
Almonds	*1766*	*471 (27%)*	1.07	0.99
Baby foods	*592*	*23 (4%)*	1.06	0.82
Brazil nuts	*622*	*271 (43%)*	1.73	0.98
Cashews	*336*	*33 (10%)*	1.14	0.99
Figs	*2067*	*618 (30%)*	1.43	0.73
Hazelnuts	*3163*	*940 (30%)*	1.23	0.83
Maize	*943*	*136 (14%)*	1.03	0.95
Other cereals	*3010*	*207 (7%)*	1.08	0.93
Other dried fruits	*1396*	*114 (8%)*	1.13	0.78
Other foodstuffs	*1604*	*303 (19%)*	1.03	0.97
Other nuts	*1131*	*158 (14%)*	1.06	1.00
Peanuts	*8929*	*1830 (20%)*	1.14	0.93
Pistachios	*4069*	*1783 (44%)*	1.10	0.97
Spices	*4698*	*1988 (42%)*	1.02	0.81
All	*34326*	*8875 (26%)*	1.24	0.93

[1] *Thirty five samples with total aflatoxins only were excluded from the regression analysis*

The slope of the equation as indicated by the linear regression coefficient is of most interest since it has to be assumed that the real intercept will be zero, i.e. a majority of samples had neither AFB1 nor total aflatoxins present. On average total aflatoxin levels

were 24% higher than AFB1 levels but with a variation of 2% to 73% for different food categories. Brazil nuts in particular but also figs seemed to have a different aflatoxin profile from the rest of the food groups. The ratio of AFB1 to total aflatoxins will vary depending on the *Aspergillus* spp. since AFB1 and AFB2 are produced by *A. flavus* and AFB1, AFB2, AFG1 and AFG2 are produced by *A. parasiticus*. The occurrence of these fungal species will vary geographically and by food commodity. As a conservative estimate, values below the LOD for total aflatoxins were set at a maximum of twice the LOD for AFB1.

Aflatoxin concentrations across food categories

Statistical descriptors for each food category with a range defined by the lower and upper bound values are presented in Table 3 for AFB1 and total aflatoxin concentrations. The number of decimals given has been adjusted for ease of reading and the food groups are sorted from high to low mean values with the three product categories of special interest at the top of the table.

The results for Brazil nuts and pistachios are clearly different with much higher mean and upper percentile values than for the other food groups. Also figs, peanuts, spices, hazelnuts and almonds have 97.5th percentile values above 2 µg/kg for AFB1 and above 4 µg/kg for total aflatoxins. There are some high maximum values for most food categories except for baby foods and maize.

Chemical food contaminants often have a lognormal distribution with most values at the low concentration end and a few high or very high values. This is obvious here with the median lower or much lower than the mean and the maximum often 10 to 100 times higher than the 95th percentile indicating a tail end of very high values.

An attempt was made to illustrate the distribution of total aflatoxin levels above LOD in all European samples tested in Figure 4. However, since the material is heavily skewed towards very low values the graph is difficult to read. Each bar in the main Figure represents an increment of 25 µg/kg and to improve readability the y-axes has been set at a maximum of 350 observations. The first bar is thus outside the scale with a total of 7,248 observations. To refine the low-level part of the graph, an insert has been produced where each bar represents an increment of 2 µg/kg. The first bar represents 4,735 observations followed by 1,035, 397, 259, and 208, respectively, for each increment of 2 µg/kg.

Table 3: Distribution statistics for different food commodities obtained in the European Union in the period 2000 to 2006 for lower to upper bound AFB1 and total aflatoxin (T) concentrations in µg/kg.

Food category	Lower to upper bound aflatoxin concentrations in µg/kg						
	Type	Median	Mean	90th %	95th %	97.5th %	Max
Pistachios	AFB1	0-0.20	16.7-16.8	27.8[1]	85.0	177.9	2625
	T	0-0.40	19.2-19.4	32.7	103.6	212.3	2680
Almonds	AFB1	0-0.20	1.36-1.46	0.78-0.80	2.00	7.2	575
	T	0-0.28	1.61-1.82	1.00	2.64	8.6	579
Hazelnuts	AFB1	0-0.16	0.85-0.95	1.40	3.00	5.6	200
	T	0-0.30	1.50-1.70	2.80	6.20	11.8	200
Brazil nuts	AFB1	0-0.20	22.0-22.2	43.6	96.9	182.6	1897
	T	0-0.40	39.3-39.6	76.24	188.8	379.3	3337
Peanuts	AFB1	0-0.10	1.80-1.93	0.60-1.00	2.34	9.8	935
	T	0-0.20	2.44-2.69	1.00-1.60	3.76	16.8	985
Spices	AFB1	0-0.20	1.33-1.46	3.10	6.60	10.9	96
	T	0-0.40	1.65-1.88	4.10	7.80	14.1	96
Figs	AFB1	0-0.15	1.25-1.36	1.20	4.80	13.0	130
	T	0-0.24	2.02-2.22	1.72-1.80	7.97	18.2	151
Other nuts	AFB1	0-0.10	1.04-1.16	0.02-0.23	0.46-1.00	1.2	385
	T	0-0.20	1.18-1.41	0.04-0.46	0.62-1.41	2.1	402
Other foodstuffs	AFB1	0-0.10	0.35-0.53	0.12-1.00	0.54-1.00	1.5	99
	T	0-0.20	0.43-0.75	0.30-1.20	0.90-2.00	2.4	99
Cashews	AFB1	0-0.10	0.29-0.42	0-0.23	0.24-1.00	1.9	36
	T	0-0.20	0.35-0.60	0-0.48	0.47-1.85	2.3	39
Other cereals	AFB1	0-0.20	0.14-0.35	0-0.50	0.10-1.00	0.7-1.0	109
	T	0-0.40	0.19-0.51	0-0.50	0.18-1.00	1.1-1.8	117
Other dried fruits	AFB1	0-0.10	0.07-0.26	0-0.40	0.04-1.00	0.3-1.0	20
	T	0-0.24	0.17-0.51	0-0.80	0.10-1.33	0.5-2.0	90
Maize	AFB1	0-0.12	0.12-0.26	0.22-0.50	0.69-0.73	1.1	8
	T	0-0.24	0.16-0.41	0.34-0.50	1.00	1.7-1.8	9
Baby foods	AFB1	0-0.02	0-0.07	0-0.10	0-0.15	0.03-1.0	0.2-1
	T	0-0.04	0-0.14	0-0.20	0-0.30	0.03-2.0	0.2-2
All foods	AFB1	0-0.15	3.32-3.46	1.30	5.50	19.8	2625
	T	0-0.30	4.28-4.53	2.00	7.90	25.9	3337

[1] *One value only is given when the lower and upper bounds are the same*

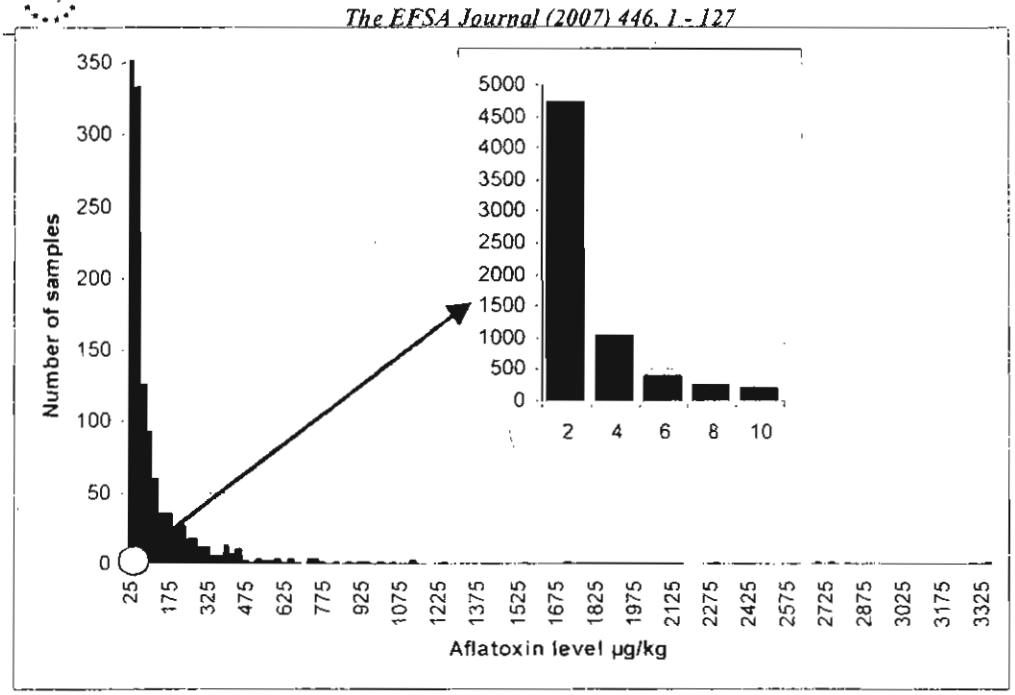

Figure 4: Histogram of the distribution of total aflatoxin levels in all samples above the limit of detection (LOD).

Of the 168 samples with levels above 200 µg/kg, 110 comprised of pistachios, 30 of Brazil nuts, 23 of peanuts, 3 of other nuts and 2 of almonds. There were five unevenly distributed very high outlier values among the 168 samples, all pistachios and Brazil nuts, which clearly biased the overall distribution. Although a rare occurrence, the heterogeneous distribution of aflatoxins with occasionally very high values is a concern that will be addressed later.

In general, for skewed data the median would give a robust and adequate measure of contamination. However the median could not be used to describe the aflatoxin data in this opinion because the majority of the observations were below the LOD. This means that the median is highly dependent on assumptions related to the LOD and to the approach to values below the LOD, with minimal impact of the actual measured data. Use of high percentile occurrence data would be relevant to assessment of acute risks, but for long term risks, the mean concentrations are more likely to be relevant.

Distribution of aflatoxins in set ranges

Using the collected data, the distribution of aflatoxin levels across food categories was further explored by analysing the proportion of samples within set ranges using MLs of 2, 4, 8, and 10 µg/kg for AFB1 and total aflatoxins as presented in Table 4.

Table 4: Distribution of levels for AFB1 and total aflatoxins (T) in defined concentration ranges across all food categories.

Food category	Proportion of samples with aflatoxins within indicated µg/kg range [1]						
	Type	<LOD	>LOD-2	>2-4	>4-8	>8-10	>10
Pistachios	AFB1	56.2%	22.7%	2.0%	2.8%	0.9%	15.4%
	T	56.2%	22.2%	2.1%	2.6%	0.8%	16.1%
Almonds	AFB1	73.3%	21.7%	1.4%	1.5%	0.3%	1.7%
	T	73.3%	20.6%	2.3%	1.1%	0.5%	2.3%
Hazelnuts	AFB1	70.3%	22.3%	4.0%	1.9%	0.4%	1.3%
	T	70.3%	17.3%	5.6%	2.7%	1.2%	2.9%
Brazil nuts	AFB1	56.4%	20.4%	2.6%	2.6%	1.0%	17.0%
	T	56.4%	18.5%	3.5%	1.9%	0.5%	19.1%
Peanuts	AFB1	79.5%	15.1%	1.3%	1.2%	0.4%	2.5%
	T	79.5%	13.8%	1.9%	1.2%	0.4%	3.2%
Spices	AFB1	57.7%	27.1%	7.4%	4.0%	1.0%	2.7%
	T	57.7%	23.7%	8.5%	5.2%	1.4%	3.4%
Figs	AFB1	70.1%	22.1%	2.3%	2.2%	0.2%	3.1%
	T	70.1%	20.6%	2.2%	2.1%	0.6%	4.4%
Other nuts	AFB1	86.0%	12.4%	0.2%	0.5%	0.1%	0.8%
	T	86.0%	11.3%	1.1%	0.4%	0.1%	1.1%
Other foodstuffs	AFB1	81.1%	17.0%	0.7%	0.3%	0.1%	0.7%
	T	81 1%	16.1%	1.5%	0.4%	0.1%	0.9%
Cashews	AFB1	90.2%	7.4%	0.9%	0.6%	0.3%	0.6%
	T	90.2%	6.5%	1.8%	0.6%	0.0%	0.9%
Other cereals	AFB1	93.1%	6.0%	0.3%	0.2%	0.0%	0.3%
	T	93.1%	5.6%	0.4%	0.4%	0.1%	0.3%
Other dried fruits	AFB1	91.8%	7.4%	0.2%	0.3%	0.1%	0.1%
	T	91.8%	7 1%	0.4%	0.3%	0.1%	0.3%
Maize	AFB1	85.6%	13.4%	0.5%	0.5%	0.0%	0.0%
	T	85.6%	12.5%	1.3%	0.5%	0.1%	0.0%
Baby foods	AFB1	96.1%	3.9%	0.0%	0.0%	0.0%	0.0%
	T	96.1%	3.9%	0.0%	0.0%	0.0%	0.0%

[1] *The EU MLs for some spices are set at 5 and 10 µg/kg for AFB1 and total aflatoxins, respectively. Different MLs also apply for some products to undergo further sorting. However, for the purpose of comparison the same ranges are used for all products.*

Although there are some slight variations between the proportion of samples within the set MLs for AFB1 and total aflatoxins, the distributions are basically the same. The number of samples with total aflatoxin levels of 4 µg/kg or less varied from 78.5% for Brazil nuts to 100% for baby foods. In fact, baby foods had no samples above 1 µg/kg. Apart from having the least number of samples at or below 4 µg/kg, Brazil nuts also had the most samples (19.1%) above 10 µg/kg. The situation for pistachios was similar with 80.5% of samples at or below 4 µg/kg and 16.1% above 10 µg/kg.

Type of sampling

Samples were selected within four types of control system: export, import, company, and market control. There were also a large number of samples that lacked information on the control system.

Company control data refer to results for products before placing them on the market as reported by companies. Import control data refer to results from border control before entering the European Union reported by Member States. Export control data refer to exports into third countries, and were in this opinion limited to a few products exported from Greece. An analysis of the findings from the four different control systems and for the unspecified monitoring is presented in Table 5 for total aflatoxin and values of less than LOD set to the LOD.

Table 5: Number of samples reported by type of control and the respective total aflatoxin levels with values less than the LOD set to the LOD (upper bound).

Type	No. of samples	Range µg/kg	Total aflatoxins µg/kg					
			Median	Mean	90th %	95th %	97.5th %	Max
Company control	*2,782 (96%)*	**0-4**	0.20	0.41	0.96	1.78	2.60	4
	2,826 (97%)	**0-8**	0.20	0.49	1.00	2.20	3.48	8
	2,849 (98%)	**0-10**	0.20	0.56	1.00	2.50	3.90	10
	2,903	**All**	0.20	2.06	1.50	3.50	8.23	426
Export control (Europe)	*70 (93%)*	**0-4**	0.40	0.43	0.40	0.40	0.43	3
	71 (95%)	**0-8**	0.40	0.50	0.40	0.40	1.08	5
	71 (95%)	**0-10**	0.40	0.50	0.40	0.40	1.08	5
	75	**All**	0.40	14.59	0.40	6.96	118.52	579
Import control	*13,507 (92%)*	**0-4**	0.20	0.40	0.72	1.20	2.10	4
	13,754 (94%)	**0-8**	0.20	0.50	1.00	1.80	3.40	8
	13,841 (95%)	**0-10**	0.20	0.55	1.00	2.00	3.98	10
	14,653	**All**	0.30	5.06	2.20	12.50	38.30	2680
Market control	*10,603 (94%)*	**0-4**	0.20	0.45	1.00	2.00	2.40	4
	10,806 (95%)	**0-8**	0.20	0.55	1.30	2.00	3.50	8
	10,878 (96%)	**0-10**	0.20	0.60	1.50	2.40	4.10	10
	11,255	**All**	0.20	4.19	2.00	5.24	16.29	2740
Unknown	*5,015 (92%)*	**0-4**	0.40	0.60	1.25	2.00	2.66	4
	5,171 (95%)	**0-8**	0.40	0.75	1.80	2.92	4.52	8
	5,202 (96%)	**0-10**	0.40	0.80	1.91	3.10	5.08	10
	5,440	**All**	0.40	4.92	2.70	7.83	24.45	3337

It should be noted that the type of product tested, and the sampling procedure, could be different between the different control systems which could influence the results.

Impact of different MLs

From Table 4 it can be seen that increasing the ML for total aflatoxins for almonds, hazelnuts and pistachios from 4 to 8 or 10 µg/kg will only have a small impact on the extra product allowed onto the market varying between 1.1-3.9% assuming that such an increase would not alter the distribution of aflatoxin concentrations in products. The distribution statistics for the different MLs to be used in the exposure assessment were calculated using data from all control systems in the European material individually for almonds, hazelnuts and pistachios. If it is assumed that all nuts exceeding the ML are not

available to the consumer, this distribution may be used to investigate the impact of increasing the ML.

Almonds

The distribution over percentiles was calculated for total aflatoxin MLs of 4, 8 and 10 µg/kg and this is shown in Figure 5.

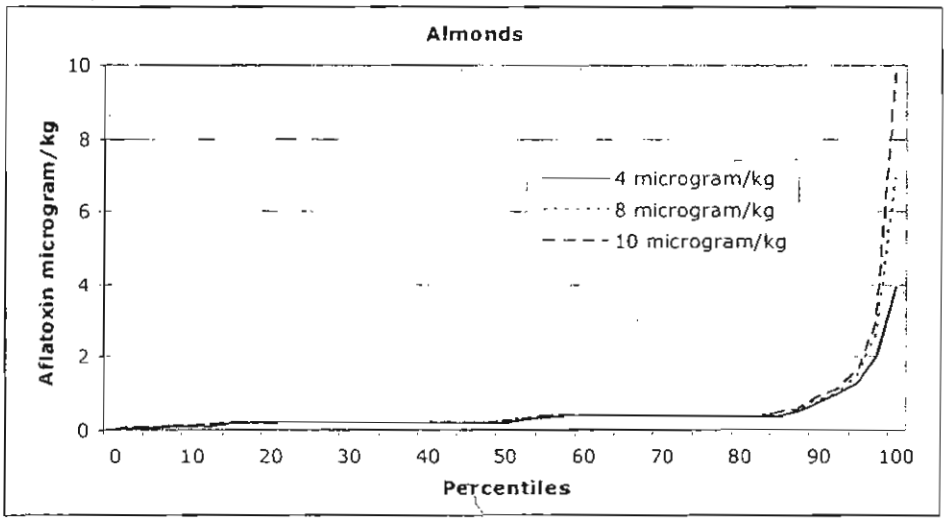

Figure 5: Illustrating the impact of three different MLs on concentrations of total aflatoxins in almonds.

The curves for almonds applying the different MLs do not start to deviate until the 90th percentile. At the 95th percentile, or for 5% of high end product, the aflatoxin concentrations are 15% higher when applying the 8 µg/kg ML and 22% higher when applying the 10 µg/kg ML compared to the 4 µg/kg ML. Selected distribution statistics are given in Table 6.

Table 6: Distribution statistics for lower bound (LB) and upper bound (UB) total aflatoxin concentrations in almonds applying three different cut off limits to the different MLs.

Total aflatoxin concentrations in µg/kg in almonds												
ML	Median		Mean		90th %		95th %		97.5th %		Max	
	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
4	0	0.20	0.18	0.40	0.60	0.79	1.26	1.30	1.90	2.00	3.92	3.92
8	0	0.20	0.24	0.46	0.70	0.80	1.45	1.50	2.60	2.60	6.90	6.90
10	0	0.22	0.29	0.50	0.79	0.90	1.50	1.59	2.99	2.99	9.80	9.80

Table 6 indicates that the mean total aflatoxin concentration is 15-33% higher when applying the 8 µg/kg ML and 25-61% higher when applying the 10 µg/kg ML compared to the 4 µg/kg ML. The larger differences arise from the lower bound values and thus from a lower base. There was only a 7% difference between AFB1 and total aflatoxins as previously shown in Table 2 so most of the above difference would consist of changes in the AFB1 concentration. The upper bound estimates for total aflatoxins are influenced by the conservative assumption that the LOD for total aflatoxins is twice the LOD for AFB1. Hence the actual increase in concentration might be less than indicated above.

Hazelnuts

The distribution over percentiles for total aflatoxin MLs of 4, 8 and 10 µg/kg is shown in Figure 6.

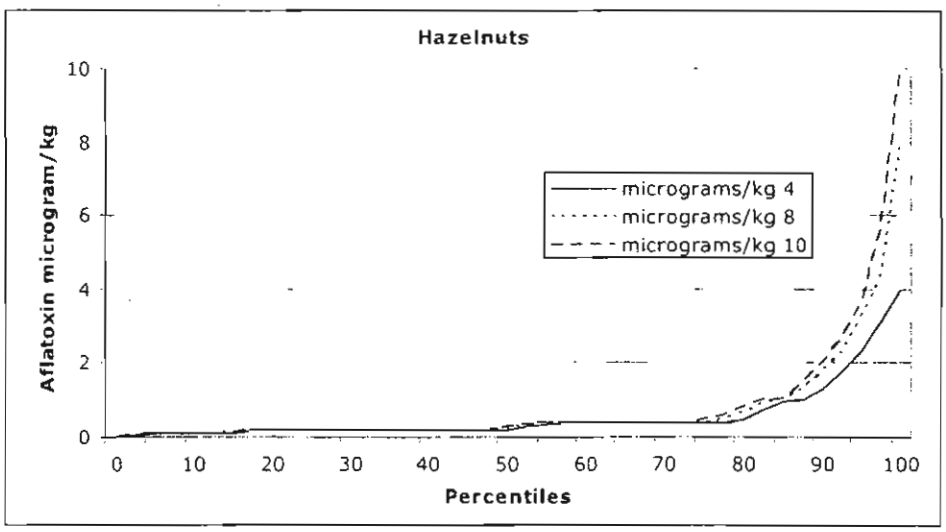

Figure 6: Illustrating the impact of three different MLs on concentrations of total aflatoxins in hazelnuts.

The curves for hazelnuts when applying the different MLs start to deviate from about the 75th percentile. At the 95th percentile, or for 5% of high end product, the aflatoxin concentrations are 39% higher when applying the 8 µg/kg ML and 57% higher when applying the 10 µg/kg ML compared to the 4 µg/kg ML. Selected distribution statistics are given in Table 7.

Table 7: Distribution statistics for lower bound (LB) and upper bound (UB) total aflatoxin concentrations in hazelnuts applying three different cut off limits.

Total aflatoxin concentrations in µg/kg in hazelnuts												
ML	Median		Mean		90th %		95th %		97.5th %		Max	
	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
4	0	0.20	0.31	0.53	1.27	1.32	2.30	2.30	3.10	3.10	4.00	4.00
8	0	0.24	0.46	0.68	1.70	1.80	3.20	3.20	4.33	4.33	8.00	8.00
10	0	0.26	0.57	0.78	1.91	2.00	3.60	3.60	5.63	5.63	10.00	10.00

Table 7 indicates that the mean total aflatoxin concentration is 26-48% higher when applying the 8 µg/kg ML and 48-84% higher when applying the 10 µg/kg ML compared to the 4 µg/kg ML. Again the larger differences arise from the lower bound values and thus from a lower base. There was a 23% difference between AFB1 and total aflatoxins so most of the above difference would consist of changes in the AFB1 concentration but there is also a contribution from other aflatoxins. The upper bound estimates are again influenced by the conservative assumption regarding the LOD for total aflatoxins.

Pistachios

The distribution over percentiles for total aflatoxin MLs of 4, 8 and 10 µg/kg MLs is shown in Figure 7.

The curves for pistachios when applying the different MLs do not start to deviate until from around the 80th percentile. At the 95th percentile, or for 5% of high end product, the aflatoxin concentrations are 75% higher when applying the 8 µg/kg ML and 120% higher when applying the 10 µg/kg ML compared to the 4 µg/kg ML. Selected distribution statistics are given in Table 8.

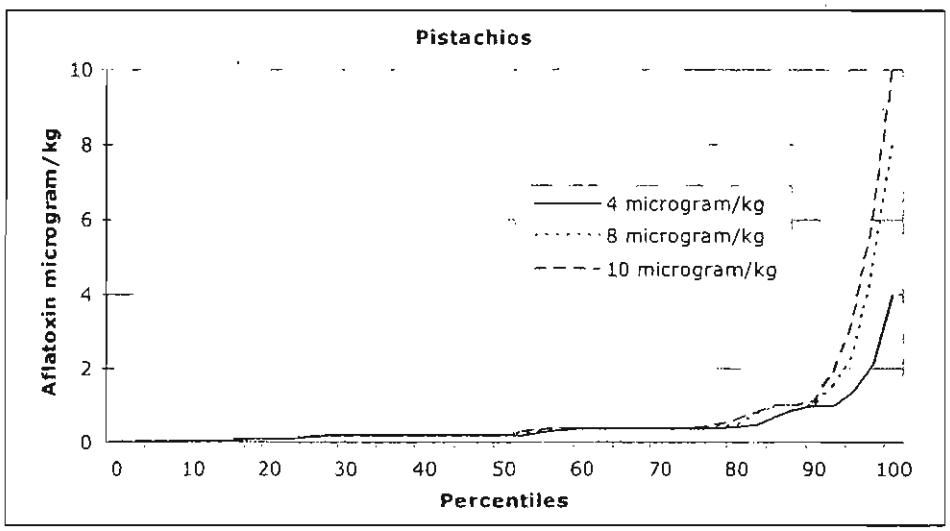

Figure 7: Illustrating the impact of three different MLs on concentrations of total aflatoxins in pistachios.

Table 8: Distribution statistics for lower bound (LB) and upper bound (UB) total aflatoxin concentrations in pistachios applying three different cut off limits.

Total aflatoxin concentrations in µg/kg in pistachios												
ML	Median		Mean		90th %		95th %		97.5th %		Max	
	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
4	0	0.20	0.20	0.44	0.60	1.00	1.38	2.00	2.12	2.12	4.00	4.00
8	0	0.20	0.37	0.61	0.93	1.60	2.41	2.41	4.70	4.70	8.00	8.00
10	0	0.20	0.46	0.69	1.02	1.84	3.03	3.03	5.70	5.70	10.00	10.00

Table 8 indicates that the mean total aflatoxin concentration is 39-85% higher when applying the 8 µg/kg ML and 57-130% higher when applying the 10 µg/kg ML compared to the 4 µg/kg ML. As for the other nuts, the larger differences arise from the lower bound values and thus from a lower base. There was only a 10% difference between AFB1 and total aflatoxins so most of the above difference would consist of changes in the AFB1 concentration. As for the other nuts, the upper bound values are affected by the conservative assumption on the LOD for total aflatoxins.

Other foods

There is no proposal to change the MLs for other foods. The current MLs have thus been used to calculate the respective contributions for those products. There are no MLs at all for what here is called other foodstuffs. The respective MLs in force were thus used to arrive at the figures in Table 9 by again assuming that product with values above the MLs would be rejected. Spices have the highest mean aflatoxin concentrations, but only small amounts of spices are consumed. Other foodstuffs, a mix of different food products excluding baby foods, have the second highest mean concentration of aflatoxins assumed

to be on the market and are likely to be consumed in higher amounts. A closer analysis of the food products at the high value end for total aflatoxins in other foodstuffs showed up several chocolate products, with a dark ready-to-eat chocolate produced in Estonia having 99 µg/kg, bitter apricot nuts imported from Turkey, some seed products imported from African countries and China, and three sausage products tested in Hungary.

Table 9: Distribution statistics for lower bound (LB) and upper bound (UB) total aflatoxin concentrations in a range of foods when applying respective legal ML as cut off points.

Total aflatoxin concentrations in µg/kg												
ML	Median		Mean		90th %		95th %		97.5th %		Max	
	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
All other nuts												
4	0.00	0.20	0.14	0.41	0.31	0.80	1.00	1.80	1.90	2.00	4.00	4.00
Maize												
4	0.00	0.24	0.12	0.37	0.30	0.50	0.92	1.00	1.50	1.52	3.71	3.71
All dried fruit												
4	0.00	0.20	0.13	0.40	0.30	0.90	0.90	1.30	1.60	2.00	4.00	4.00
Spices												
10	0.00	0.40	0.89	1.13	3.10	3.10	5.00	5.00	7.06	7.06	10.00	10.00
Other foodstuffs												
All	0.00	0.20	0.43	0.75	0.30	1.20	0.90	2.00	2.39	2.39	99.00	99.00

Information from tables 6 to 9 is used as occurrence data for the exposure calculations (see chapter 3.5 and 3.6).

It has to be again pointed out that all the analyses so far have been undertaken with the assumption that the control system is fully effective. If, as is likely, this is not the case the contribution to the overall aflatoxin burden from other foodstuffs as shown above, where no ML is applied, will be significantly overestimated since contributions from the other food categories including nuts would increase significantly. The sensitivity analysis below will address this issue in more detail.

Effectiveness of import controls

Ready-to use product testing, whether for export, import or market control purposes, cannot result in removal of all products above the ML from the market. However, it will act as a deterrent and thus encourage introduction of production quality assurance systems with the capacity of reducing the overall contamination pressure.

To assess the efficiency of the import control system in diverting product above the ML, a comparison of test results from the import control and the random control in the form of market and company testing was undertaken on almonds, hazelnuts and pistachios, products that are almost exclusively imported to the EU. Results are shown in Table 10.

To assess the efficiency of the import control system in diverting product above the ML, a comparison of test results from the import control and the random control in the form of market and company testing was undertaken on almonds, hazelnuts and pistachios, products that are almost exclusively imported to the EU. Results are shown in Table 10.

Table 10: Number of samples reported by type of control and the respective total aflatoxin levels for almonds, hazelnuts and pistachios.

Type	No. of samples	Range µg/kg	Total aflatoxins µg/kg					
			Median	Mean	90th %	95th %	97.5th %	Max
Import control	*3,434 (84%)*	**0-4**	0.20	0.45	1.00	1.90	2.80	4
	3,554 (87%)	**0-8**	0.20	0.64	1.40	3.16	4.90	8
	3,599 (89%)	**0-10**	0.20	0.74	1.70	3.80	6.21	10
	4,068	**All**	0.40	12.22	14.20	48.17	119.3	2680
Random control	*2,522 (89%)*	**0-4**	0.20	0.40	0.90	1.75	2.50	4
	2,565 (91%)	**0-8**	0.20	0.49	1.00	2.00	3.40	7.40
	2,601 (92%)	**0-10**	0.20	0.61	1.20	2.70	5.10	10
	2,820	**All**	0.20	10.47	5.11	28.71	111.05	2278

Eleven percent of the three nut products on the EU market still have total aflatoxin levels above 4 µg/kg. Rather than a calculated mean of 0.40 µg/kg should the import control be fully effective, the actual mean for product on the market was 10.47 µg/kg. It might be argued that the random market testing provides a further safety net for consumers in that some of the product above the ML will be condemned and destroyed. However, it is impossible to draw any conclusions on products exceeding the MLs on the market from the available data.

Sample heterogeneity was checked on 1,024 samples with multiple testing, that is two or more samples were taken from the same food lot and tested separately. In 88 of the cases all sub-samples produced the same result. Among the other samples, the largest variation recorded was for a pistachio sample from the import control with three sub-samples showing <0.2, 235 and 1,031 µg/kg, respectively. The maximum and mean calculated from the sub-samples that differed in aflatoxin concentration are illustrated in Figure 8.

would be rejected. Spices have the highest mean aflatoxin concentrations, but only small amounts of spices are consumed. Other foodstuffs, a mix of different food products excluding baby foods, have the second highest mean concentration of aflatoxins assumed to be on the market and are likely to be consumed in higher amounts. A closer analysis of the food products at the high value end for total aflatoxins in other foodstuffs showed up several chocolate products, with a dark ready-to-eat chocolate produced in Estonia having 99 µg/kg, bitter apricot nuts imported from Turkey, some seed products imported from African countries and China, and three sausage products tested in Hungary.

Table 9: Distribution statistics for lower bound (LB) and upper bound (UB) total aflatoxin concentrations in a range of foods when applying respective legal ML as cut off points.

Total aflatoxin concentrations in µg/kg												
ML	Median		Mean		90th %		95th %		97.5th %		Max	
	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
All other nuts												
4	0.00	0.20	0.14	0.41	0.31	0.80	1.00	1.80	1.90	2.00	4.00	4.00
Maize												
4	0.00	0.24	0.12	0.37	0.30	0.50	0.92	1.00	1.50	1.52	3.71	3.71
All dried fruit												
4	0.00	0.20	0.13	0.40	0.30	0.90	0.90	1.30	1.60	2.00	4.00	4.00
Spices												
10	0.00	0.40	0.89	1.13	3.10	3.10	5.00	5.00	7.06	7.06	10.00	10.00
Other foodstuffs												
All	0.00	0.20	0.43	0.75	0.30	1.20	0.90	2.00	2.39	2.39	99.00	99.00

Information from tables 6 to 9 is used as occurrence data for the exposure calculations (see chapter 3.5 and 3.6).

It has to be again pointed out that all the analyses so far have been undertaken with the assumption that the control system is fully effective. If, as is likely, this is not the case the contribution to the overall aflatoxin burden from other foodstuffs as shown above, where no ML is applied, will be significantly overestimated since contributions from the other food categories including nuts would increase significantly. The sensitivity analysis below will address this issue in more detail.

Effectiveness of import controls

Ready-to use product testing, whether for export, import or market control purposes, cannot result in removal of all products above the ML from the market. However, it will act as a deterrent and thus encourage introduction of production quality assurance systems with the capacity of reducing the overall contamination pressure.

It was also not possible to obtain detailed pre-export data for **pistachios** from Iran which is the largest pistachio nut producer in the world. Only a pre-print of a journal article dealing with the incidence of aflatoxins in Iranian pistachios intended for export into the European Union was made available. A total of 3356 pistachio nut samples were collected between March 2002 and February 2003, divided into 10,068 sub-samples according to EU requirements and analysed for aflatoxin contamination. The number of samples that exceeded the EU MLs for AFB1 and total aflatoxins is given as 15.9 and 13.6%, respectively (Cheraghali *et al.*, 2007). Unfortunately, the ranges used for the further evaluation of the concentration of total aflatoxins (<LOD, LOD-5, 5-15, 15-50, 50-500, >500 µg/kg) differ significantly from those applied in this assessment making a reliable appraisal of their results for the purpose of this EFSA assessment impossible.

A total of 553 individual results of pre-export checks of pistachios produced in Turkey in 2005 and 2006 could be accessed. Moreover, Turkey provided 6204 results for aflatoxins in **hazelnuts** analysed before export in 2005 and 2006. Turkey is the greatest exporter of hazelnuts and also a major producer and exporter of pistachios into the EU.

Results from Turkish pre-export controls on hazelnuts and pistachios 2005/2006

The results of the Turkish pre-export controls were grouped into various concentration ranges (0-4, 4-8, 8-10, and >10 µg/kg) in order to estimate the impact on aflatoxin occurrence in hazelnuts and pistachios in response to proposed change of the MLs. Table 11 gives an overview of the percentage distribution.

Table 11: Distribution of AFB1 and total aflatoxin (T) levels in defined concentration ranges for Turkish hazelnuts and pistachios tested before export in 2005/2006.

Food Category	Proportion of samples with total aflatoxins (T) within indicated ranges (µg/kg)						
	Type	<LOD	>LOD-2	>2-4	>4-8	>8-10	>10
Hazelnuts	AFB1	85.9%	12.2%	0.9%	0.4%	0.1%	0.6%
	T	83.2%	11.7%	2.9%	0.8%	0.2%	1.1%
Pistachios	AFB1	76.4%	7.2%	3.1%	4.9%	2.0%	6.2%
	T	76.1%	5.6%	3.8%	4.2%	1.8%	8.5%

As can be seen, 83.2% of all hazelnut lots tested were below the limit of detection of 0.20 µg/kg and 97.8% of all hazelnut consignments were below the current EU ML of 4 µg/kg for total aflatoxins. Another 0.8% and 0.2% were between 4-8 and 8-10µg/kg, respectively. A total of 1.1% of the hazelnut samples tested before export in 2005/2006 exceeded 10 µg/kg for total aflatoxins.

A somewhat different situation can be observed for pistachios. Although the total number of pistachio export lots tested (n=553) was considerably lower than hazelnuts (n=6204), the number of samples that exceeded the current ML for total aflatoxins was substantially higher. Almost 15% of the pistachio lots tested in Turkey before export in 2005/2006 were not compliant with the current EU Regulation. Moreover, 8.5% of the pistachio lots

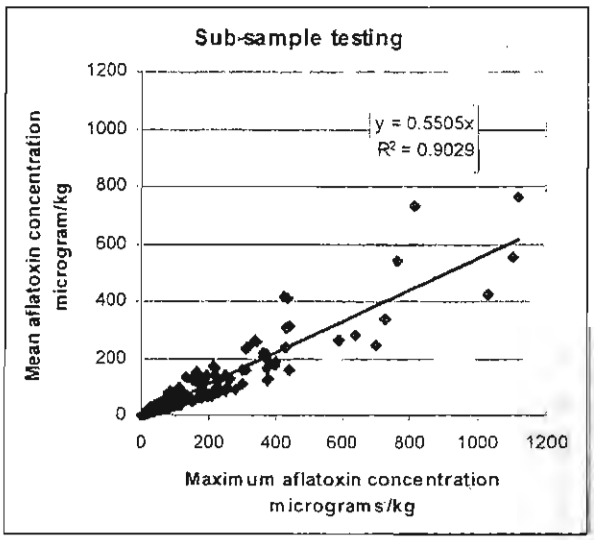

Figure 8: Comparison of total aflatoxin maximum and mean concentrations in testing of multiple sub-samples from one food lot.

The average maximum is almost double the average mean in repeat testing of multiple sub-samples confirming the earlier described heterogeneity in aflatoxin distribution in contaminated food products.

3.3 Results from pre-export controls

In order to estimate whether increased MLs for total aflatoxins would have an impact on the contamination of nuts that are intended for export to the European Union it was attempted to obtain occurrence data from third countries for almonds, hazelnuts and pistachios analysed before export as part of due diligence of the producers and compliance check with the current EU regulation.

According to the Almond Board of California, the USA is the largest producer of **almonds** worldwide. The crop for 2006 is envisaged to be 476,000 tonnes, and approximately one third of these will be exported to the European Union. Thus, almost 97% of almonds imported to the EU originate from the USA. To date, there is no mandatory outgoing control for aflatoxin monitoring. Most of the controls are undertaken voluntarily by the industry or recommended through the Almond Board of California. For example, a voluntary aflatoxin sampling programme was initiated by the Almond Board of California and started as a pilot programme from the beginning of the 2006 crop in September 2006 with five processors. There is proposed legislation to increase the percentage of inedible nuts removed from the raw ingredient, and for a voluntary aflatoxin sampling plan (DG (SANCO)/8300/2006 – MR Final). Hence, no significant numbers of occurrence data are currently available of almonds produced in the USA and checked before export for aflatoxins.

tested before export was exceeding 10 µg/kg, in comparison to only 1.1% of the hazelnut samples. On the other hand, 76.1% of the pistachio lots were below the limit of detection of 0.20 µg/kg for total aflatoxins.

Detailed histograms illustrating the different distributions of total aflatoxin occurrence data in Turkish hazelnuts and pistachios analysed before export in 2005/2006 are depicted in Figure 9.

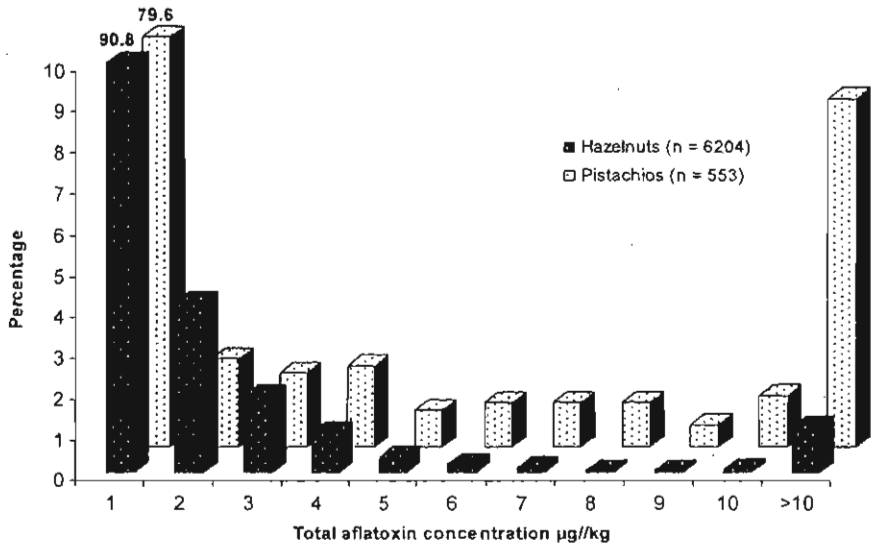

Figure 9: Frequency histograms for total aflatoxins in Turkish hazelnuts and pistachios tested before export 2005/2006.

The LOD for AFB1 in hazelnuts and pistachios was given by the Turkish authorities as 0.10 µg/kg. Taking into account that AFB1 in hazelnuts and pistachios amounts on average to around 75% of total aflatoxins, for a worst case scenario the limit of detection for total aflatoxins was set as twice (0.20 µg/kg, upper bound) the LOD for AFB1. For comparison, a second evaluation was performed for which the LOD was set to zero (lower bound). Based on these assumptions, distribution statistics were calculated for the two types of nuts. Table 12 presents these descriptions for AFB1 and total aflatoxins as determined in the Turkish pre-export controls performed in 2005/2006.

Table 12: Distribution statistics for hazelnut and pistachio pre-export controls 2005/2006 for lower to upper bound AFB1 and total aflatoxin (T) concentrations in µg/kg.

Food category	Type	Lower bound/upper bound aflatoxin concentrations (µg/kg)					
		Median	Mean	90th %	95th %	97.5th %	Max
Hazelnuts	AFB1	0.00-0.10	0.36-0.44	0.45	0.85	1.55	218
	T	0.00-0.20	0.71-0.87	0.84	2.05	3.59	243
Pistachios	AFB1	0.00-0.10	3.31-3.39	6.07	17.3	36.6	119
	T	0.00-0.20	4.79-4.94	8.60	32.6	52.8	164

Only one value is given if the lower bound and upper bound values are the same

The change in the concentrations of total aflatoxins at various hypothetical MLs is shown in Table 13.

Table 13: Number of hazelnut and pistachio samples and the respective total aflatoxin levels (lower bound/upper bound) tested before export assuming various hypothetical MLs.

Type	No. of samples	Range µg/kg	Lower bound/upper bound concentration of total aflatoxins (µg/kg)					
			Median	Mean	90th %	95th %	97.5th %	Max
Hazelnuts	6071 (98%)	0 - 4	0.00 / 0.20	0.18 / 0.35	0.57	1.50	2.24	4.0
	6123 (99%)	0 - 8	0.00 / 0.20	0.23 / 0.40	0.68	1.62	2.61	7.8
	6134 (99%)	0 - 10	0.00 / 0.20	0.24 / 0.41	0.70	1.69	2.75	9.9
Pistachios	473 (86%)	0 - 4	0.00 / 0.20	0.19 / 0.37	0.39	1.69	2.88	3.9
	496 (90%)	0 - 8	0.00 / 0.20	0.47 / 0.64	1.53	3.82	5.71	7.4
	506 (92%)	0 - 10	0.00 / 0.20	0.64 / 0.81	2.28	5.19	7.61	9.2

Only one value is given if the lower bound and upper bound values are the same

Due to the fact that the concentrations of total aflatoxins in 83.2% of the hazelnut and 76.1% of the pistachio samples were below the limit of detection, the lower bound and upper bound median levels will not change if the ML was raised from 4 to 8 or 10 µg/kg.

For hazelnuts, a possible shift for total aflatoxins from the current EU level of 4 µg/kg to 8 or 10 µg/kg would result in an increase of the mean concentration (upper bound) from 0.35 to 0.40 or 0.41 µg/kg, respectively. The corresponding lower bound values would increase from 0.18 to 0.23 or 0.24 µg/kg. Thus, a possible increase in the ML from 4 µg/kg to 8 or 10 µg/kg would result in an increase of the mean levels for total aflatoxins in hazelnuts by approximately 20-30%, provided the same extent of good agricultural practice would be applied during production. A similar increase can be observed for the 90th, 95th and 97.5th percentiles. In these cases, lower bound and upper bound values remain the same. Figure 10 shows detailed histograms of the frequency distribution of total aflatoxins in Turkish hazelnuts before export in dependence of the two years of analysis.

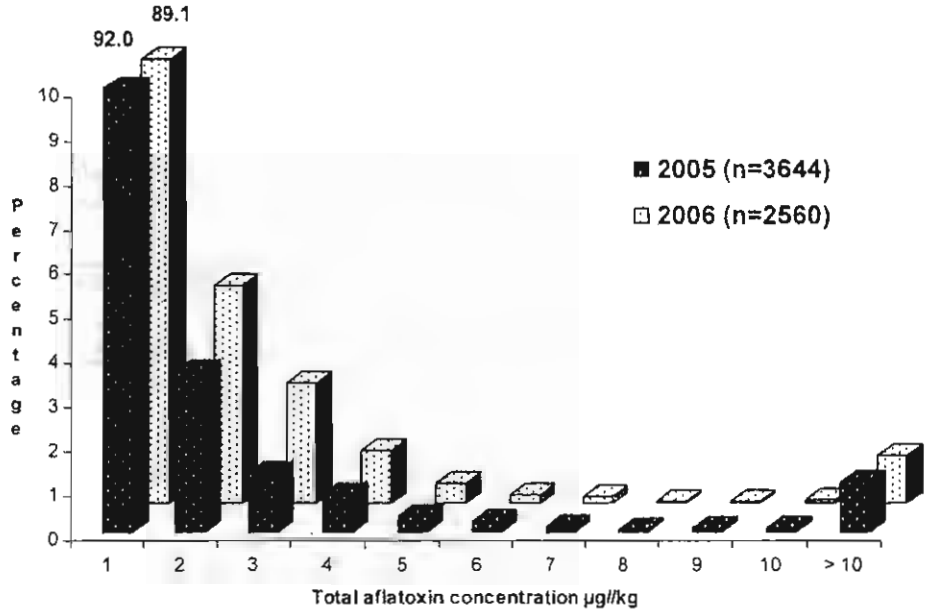

Figure 10: Frequency histograms for total aflatoxins in Turkish hazelnuts tested before export in the years 2005 and 2006.

As can be seen, irrespective of the year the frequency distribution of the samples for the concentration range between >4 and 10 µg/kg is very similar.

Compared to hazelnuts, the situation for pistachios is clearly different as an increase of the ML from 4 µg/kg to 8 or 10 µg/kg would affect the mean levels, 90th, 95th and 97.5th percentiles significantly as can be seen from Table 13. While a possible increase of the ML from 4 µg/kg to 8 or 10 µg/kg would raise both the lower bound and upper bound mean level by a factor of 2-3, the 90th, 95th and 97.5th percentiles would increase by factors between 2 and 6. Thus, the impact of higher ML seems to be more pronounced for pistachios than for hazelnuts.

In contrast to hazelnuts, the histograms of the frequency distribution of total aflatoxins in pistachios show more variation for the contamination range between >4 and 10 µg/kg as is depicted in Figure 11.

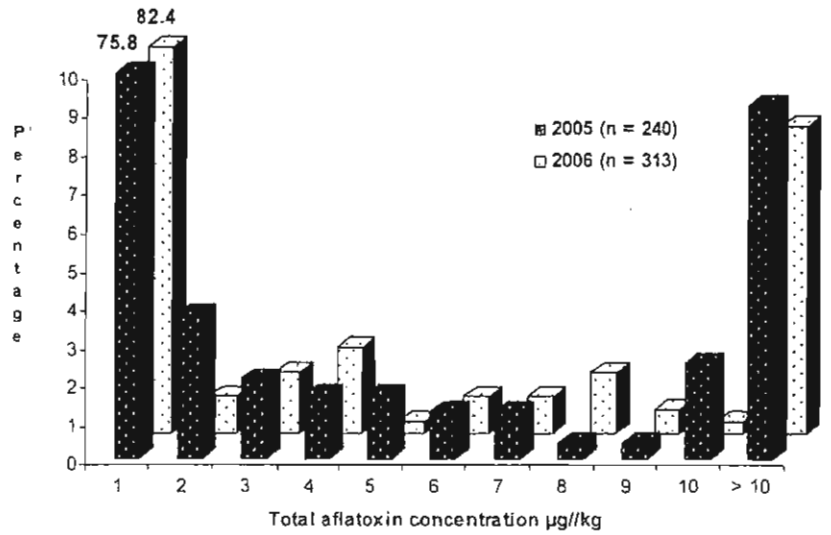

Figure 11: Frequency histograms for total aflatoxins in Turkish pistachios tested before export in the years 2005 and 2006.

3.4 Conclusions from the evaluation of aflatoxin occurrence data

In total, 34,326 analytical results for occurrence of aflatoxins in various foodstuffs submitted by 20 Member States as well as 6,762 results from pre-export controls submitted by Turkey, each in response to a call for information by the European Commission were considered for this assessment. The samples showed a broad range of contamination generally with a log-normal distribution. Overall, 74% of all samples were below the LOD, with the LOD varying considerably between laboratories. For the statistical evaluation, the LOD was either entered as the actual numerical value (upper bound) or replaced by zero (lower bound). The impact of treating the varying LODs as upper bound or lower bound values will of course be greater at low levels of the concentration spectrum and thus especially influence calculations of the contribution from other low contaminated food products.

The CONTAM Panel also received data relating to concentrations of AFM1 in commercial milk samples. For almost all of these data, the values for AFM1 concentrations were below 0.05 µg/kg and taking into account the lower carcinogenic potency of AFM1 the Panel did not consider these data further.

The highest total aflatoxin levels were found in pistachios and Brazil nuts. These two food commodities also showed the highest percentage of lots which did not comply with the current EU MLs. Statistical evaluation of all samples with aflatoxin levels above the LOD indicated that AFB1 generally is the dominating aflatoxin. On average, total aflatoxin concentrations were only 24% higher than AFB1 levels with a variation of 2-73% for different food categories. For a conservative estimate, values below the LOD for

total aflatoxins were set as a maximum of twice the LOD of AFB1 when calculating upper bound values. Brazil nuts and figs seem to have a different aflatoxin profile compared to the other food categories. It was also confirmed by analysing multiple sub-samples that sample heterogeneity is a confounding factor in aflatoxin evaluations with a more than 1000-fold difference detected between sub-samples in the worst case.

Special emphasis was given to the evaluation of the occurrence of aflatoxins in almonds, hazelnuts and pistachios. Testing of almonds revealed aflatoxin contamination levels of up to 579 µg/kg. Hazelnut testing did not demonstrate as high a maximum as for almonds, but on the other hand there were more test results at the high end as reflected in higher concentrations in the 90th percentile and above. Results from testing of pistachios indicated considerable contamination at the high end. Some very high values were found with a maximum of 2,625 µg/kg in one lot.

In relation to the question about estimating the **impact of a change of the MLs** for total aflatoxins from the current EU level of 4 µg/kg to 8 or 10 µg/kg for ready-to-eat almonds, hazelnuts and pistachios and derived products, the respective occurrence data were truncated at the relevant concentrations and statistical parameters for each concentration range were calculated.

A change in the current ML for **almonds** from 4 to 8 or 10 µg/kg would add another 1.1% or 1.6% of lots (Table 4) as compliant and would result in an increase of the mean level for total aflatoxins from 0.40 to 0.46 or 0.50 µg/kg for upper bound and from 0.18 to 0.24 or 0.29 µg/kg for lower bound values (Table 6).

A change in the current ML for **hazelnuts** from 4 to 8 or 10 µg/kg would add another 2.7% or 3.9% of lots (Table 4) as compliant and would result in an increase of the mean level for total aflatoxins from 0.53 to 0.68 or 0.78 µg/kg for upper bound and from 0.31 to 0.46 or 0.57 µg/kg for lower bound values (Table 7).

A change in the current ML for **pistachios** from 4 to 8 or 10 µg/kg would add another 2.6% or 3.4% of lots (Table 4) as compliant and would result in an increase of the mean level for total aflatoxins from 0.44 to 0.61 or 0.69 µg/kg for upper bound and from 0.20 to 0.37 or 0.46 µg/kg for lower bound values (Table 8).

Expressed as percentages, the above increases in the mean levels for total aflatoxins would seem to be high. However, it is notable that even the upper bound concentrations remain below 1 µg/kg of nuts.

The mean concentrations are greatly influenced by the relatively few samples at the higher end of the distribution range. In practice, it is unlikely that the controls are fully effective, and occasional consumption of nuts contaminated at the very much higher (non compliant) levels sometimes reported would further increase the mean contaminant levels and hence reduce the impact of increasing the ML.

Although a comparison of the total aflatoxin distribution in the data sets of the **pre-export controls** and the results provided by the Member States indicates that the fraction of samples that comply with the current EU ML is similar, their values differ to a certain extent. In general, the derived statistical values for the Turkish pre-export controls are lower than the results from the Member States. This might be due to the different number of lots tested as well as the different analytical methodologies applied. While the occurrence data reported from the 20 Member States were generated in numerous laboratories which apply methods with considerably different limits of detection, the data set from Turkey seems to be more homogenous because of the few laboratories involved with similar analytical methods and comparable limits of detection. However, irrespective of the actual contamination level, the pre-export data clearly indicate that raising the MLs will have a greater impact on exports of pistachios than of hazelnuts.

The Panel noted the very high aflatoxin concentrations recorded in Brazil nuts and pistachios from products on the market. The presented statistical calculations (see chapter 3.2, Tables 3 and 4) revealed that reducing the number of such high value samples would have more impact on total human exposure than the change of the MLs from 4 to 8 or 10 µg/kg. Therefore, it is recommended to improve pre-export and production quality assurance systems.

3.5 Food consumption data

Data description

In support of the aflatoxin review, the European Commission asked Member States to provide data on nut consumption. Nut consumption data were submitted from 8 Member States. The surveys undertaken by the Member States differed in methodology, age-groups used and food classifications. Furthermore, the Member States reported the data in aggregated and non standardised formats. Hence, there is high uncertainty in interpreting variation of food consumption between Member States.

Data provided by the Member States on nut consumption are mainly based on individual consumption data collected via food surveys. Submissions of Estonia and Hungary could not be used because only aggregated or non individual measured data were submitted.
All countries submitted data for hazelnuts, pistachios and almonds. Data for other foods that could contain aflatoxins were not submitted from all countries or were incomplete. To consider exposure from foods other than nuts in estimating the influence of changes to the MLs for hazelnuts, pistachios and almonds information on exposure from other food sources is also required. For this purpose the CONTAM Panel used the diets from the GEMS/Food Consumption Cluster Diets database of the FAO/WHO (2006). Such data allow the extrapolation of the mean exposure to all populations of European Member States including those who did not submit any data.

Reports from the Member States did not include the same age-groups. The analyses that were performed related to two groups, children and adults, with age differences within these two groups.

Information on nut consumption for vegetarians was provided only from the UK and France. The data from France only gave aggregated consumption which cannot be used for estimating changes of exposure under different assumptions relating to the MLs. It is uncertain whether or not the sampling in the relatively small UK vegetarian survey is representative of all British vegans and vegetarians. Moreover, the data are based on a food frequency method, which is considered less accurate than food record methods. Nevertheless, these were the only datasets available for vegans and vegetarians and these were therefore included in the exposure assessments.

Another important point to consider is the lack of European harmonisation in the methodology for collecting data within food surveys for risk assessments, i.e. different methods would influence the estimates of long-term exposure. Although it is well known that a 24h-recall with two or less days will yield over-estimations for long-term mean exposures of rarely consumed foods (e.g. in the Spanish data), but the Panel decided not to exclude any data on the basis of the food consumption methodology.

All submitted data, with the exception of the UK vegetarian survey, were from cross-sectional food surveys which are representative of the age-groups within the population. Other European data sources from cohort studies used for nutritional epidemiology (e.g. EPIC) could not be considered. These data were not considered appropriate for the risk assessment because they do not represent the structure of populations.

Table 14 provides information on the types of food survey data used in the exposure assessment.

Table 14: Main characteristics of the National Food Surveys used in the assessment.

Country	Reference	Survey methodology	Year of survey	Population subgroups included in the assessment	Food classification/ aggregation reported[1]	Sampling
UK, adults	Henderson *et al.*, 2002	7 days weighing protocol	2000	19-64 years (N=1,724)	including and not-including ingredients	representative for UK adults
UK, children	Gregory, *et al.*, 2000	7 days weighing protocol	1997	4-18 years (N=1701)	including and not-including ingredients	
UK, young children	Gregory, *et al.*, 1995	4 days weighing protocol	1992	1.5-1.5 years (N=1675)	including and not-including ingredients	
UK, children	Info. to EFSA FSA, 2006	Self-administered questionnaire incl. food frequency	2006	4-14 years (N=227)	ingredients not asked	-
UK vegetarian	Info. to EFSA FSA, 2006	Self-administered questionnaire incl. food frequency.	2006	all (N=270)	ingredients not asked	not clear whether the sample is representative
Spain, adults	AESA, 2006	3 day food record	-	17-60 years (N=1,060)	food as eaten	representative for Spain adults
Spain, children	AESA, 2006	3 day food record	-	7-12 years (N=903)	food as eaten	representative for Spain children
Germany, adults	Mensink *et al.*, 1998	"dietary history" for one month using DISHES	1998	18+ (N=4,030)	including ingredients	representative for German adults
Germany, children	Banasiak *et al.*, 2005	2x3 days weighing/ estimating protocols	2003	2-5 years (N=475)	including ingredients	representative for German children
Hungary, adults	Information to EFSA	-	-	18+ (N=1,179)	nut like products and oily seed aggregated to one single group	-
Estonia	Information to EFSA	Consumption statistics by households	2005	Entire population	food as eaten - rough categories	-
Ireland, adults	IUNA, 2001	7-day estimated food record	1997 -1999	18-64 years (N=985)	including ingredients	representative for Irish adults
Ireland, children	IUNA, 2005	7-day weighing record	2003 -2004	5-12 years (N=594)	including ingredients	representative for Irish children
France, adults	Volatier, 2000	Precoded 7-day estimated record	1998 -1999	15+ (N=1,474 without underreporting)	including ingredients	representative for France adults
France, children	Volatier, 2000	Precoded 7-day estimated record	1998 -1999	3-14 years (N=1,018)	including ingredients	representative for France children
France, vegetarians	Leblanc *et al.*, 2000	Precoded 7-day estimated record	2000	> 15 year (N=145)	not including ingredients	representative for the French vegetarian population
Sweden, children	Barbieri *et al.*, 2006	Open/ estimated food diary over 4 consecutive days	2003	4, 8 and 12 years	-	-

[1] *not necessarily the same as in the original survey.*

GEMS/ Food database

The GEMS/Food Consumption Cluster Diets are based on national food balance sheets of annual food production as well as import and export for individual countries, aggregated into clusters according to similar consumption behaviour. The main advantage of the data is the good comparability between different countries because the same methodology and standardised food classification system of the Codex Alimentarius were used. However, data from food balance sheets do not give information on consumption at the individual level, so only a "per capita" mean consumption amount of a population can be derived. Information on high percentiles of the population and on selected population subgroups (age-groups, vulnerable subgroups) cannot be derived from these data. Table 15 lists all the relevant clusters for this opinion.

Table 15: Composition of GEMS/Food Consumption Cluster Diets that include European Member States.

Cluster B	Cluster E		Cluster F
Cyprus	Austria	Luxembourg	Estonia
Greece	Belgium	Malta	Finland
Israel	Croatia	Netherlands	Iceland
Italy	Czech Republic	Poland	Latvia
Lebanon	Denmark	Slovakia	Lithuania
Portugal	France	Slovenia	Norway
Spain	Germany	Switzerland	Sweden
Turkey	Hungary	United Kingdom	
United Arab Emirates	Ireland		

In Table 16, the mean consumption amounts from the GEMS/Food database for the three nuts (almonds, hazelnuts and pistachios) together with consumption figures for other foods relevant for assessment of total dietary exposure are listed.

Table 16: Consumption of nuts and other food items relevant for the exposure assessment according to the GEMS/ Food Consumption Cluster Diets database in gram per day (mean of all population, ingredients included).

	Cluster B	Cluster E	Cluster F
Almonds	1.9	1.0	0.8
Hazelnuts	2.1	1.3	0.3
Pistachios	0.7	0.3	<0.1
Consumption of other food items	239.5	110.3	67.5
- Other nuts (including groundnuts shelled, except coconuts)	6.2	5.0	1.5
- Maize[7] (including oil, sweet corn, kernels and pop corn)	150.6	39.9	14.8
- Oilseeds[8] (except groundnuts)	62.1	58.1	38.0
- Dried fruits (including coconuts)	19.5	5.5	12.1
- Spices	1.1	1.8	1.1

The definition of exposure from other food items in this opinion includes nuts other than almonds, hazelnuts and pistachios, maize, oilseeds, dried fruits and spices. The category "oilseeds" in the submitted occurrence data contains food commodities like sunflower seeds, sesame seeds, pumpkin seeds and poppy seeds. To match these values with the GEMS/Food classification the consumption values for groundnuts in GEMS/Food were excluded from the "oilseeds" category and added to the category "other nuts".

Although some Member States reported a number of results for aflatoxins in "other foods" and "other cereals" (e.g. rice, cacao, tea) these categories were not included in the assessment. The reason for this exclusion is the scarcity of submitted results for such food groups and literature reports showing that their contamination is negligible (EC, 1997b) Nevertheless the Panel recommended more extensive analyses of aflatoxin levels in other foodstuffs so that unknown contributors to aflatoxin exposure are not neglected.

From Table 16, it is clear that the consumption of other products (especially maize and oilseeds) is considerably greater than the consumption of hazelnuts, almonds and pistachios. However, the exposure assessment needs to consider and analyse data for the consumption of individual foods in relation to their levels of aflatoxin contamination.

[7] The value for consumption of maize corresponds to a calculated value obtained from food balance sheet transformed product back to raw product using the converting factors of the FAO (maize is the maximum of 1.2 x maize flour or 16.7 x maize germ and 0.2 x beer of maize; germ maize is the maximum of germ maize and 2.2 x maize oil.

[8] This food category takes into account declared food balance sheet for oil using different factors, according to the type of seed, in converting back to the raw seed (linseed, melon, sunflower, mustard, poppy, rape, safflower, palm, olive and sesame).

The GEMS/Food Consumption Cluster Diets database only provided overall means. Therefore, population subgroup values could not be derived. However, such data have been obtained from the national food surveys for almonds, hazelnuts and pistachios.

National surveys

For the exposure assessment three subgroups should be considered: adults, children and subgroups with higher nut consumption which might include vegans and vegetarians. In the food surveys, no information was provided regarding patients with hepatitis B infection and this subgroup was not included. There is no indication that their nut consumption would be any different to other subgroups.

All figures in the tables are given in gram per day. When the fraction of consumers of one food item was too small, so that it was not possible to derive a value for the upper percentiles, then the highest value was taken as the upper percentile estimate.

For all tables it should be kept in mind that the age groups sometimes differ as well as the survey methodology. Because the food classification system used for collecting and reporting data can affect the estimates, this information is given in a separate column.

Adults

Table 17 shows that the proportions of almond consumers differ widely between countries. Some of the differences can be explained by the food classification. The proportion of consumers eating almonds (1.5% unshelled and 5.7% shelled) in Spain was lower than that for other countries, and this is because products made of almonds (e.g. "Turrones and mazapanes", cakes, chocolate or breakfast cereals) were not included in the Spanish food category for almonds. Therefore values for the proportion of consumers eating almonds in Spain are an underestimate.

Table 17: Consumption figures of almonds taken from food surveys of the Member States and GEMS/Food Consumption Cluster Diets database for adult population.

Country	All population				Consumers only				Remark
	N	Mean (g/day)	95th %	97.5th %	%	Mean (g/day)	95th %	97.5th %	
GEMS/ Food Cluster B		1.9							ingredients included
GEMS/ Food Cluster E		1.0							
GEMS/ Food Cluster F		0.8							
Spain	1,060	0.2	2.6	3.1	1.5	9.8	21.9	24.2	unshelled, ingredients not included
Spain	1,060	0.5	4.9	5.8	5.7	8.6	21.2	23.7	shelled, ingredient not included
Germany	4,030	0.4	2.0	-	29.0	1.3	5.4	-	ingredients included
Ireland	1,379	0.1	-	-	10.5	0.8	-	5.9	ingredients included
France	1,474	0.5	2.1	-	29.8	1.6	3.7	-	ingredients included
United Kingdom	1,724	0.1	-	-	2.0	3.3	-	15.9	ingredients not included
United Kingdom	1,724	0.5	-	-	32.0	1.6	-	8.3	ingredients included

It is often reported that the amount of rarely consumed foods tends to be overestimated in food surveys. This is due to the limited number of survey days which does not allow full exclusion of intra-individual effects. Thus, the estimated amounts of almonds consumed in Spain (3-day survey) are above the values of other countries and the percentage of consumers is lower. It cannot be concluded whether this difference is only an effect of the number of consumers or if consumers in Spain eat more almonds than in other countries. These values are also likely to be affected by the exclusion of ingredients from the survey because, as seen in the UK data, including ingredients increases the proportion of consumers and decreases the amounts consumed per consumers only.

The estimates of the GEMS/Food Consumption Cluster Diets database are quantitatively similar or slightly higher than the national survey values for mean consumers of almonds including ingredients. This can be explained by the different types of data collection used and the effects of other countries in the respective clusters.

Table 18 shows the same pattern for hazelnuts as for almonds for the Spanish data. German adults had the highest proportion of hazelnut consumers in the population (37.6%) and, except for the Spanish and UK data excluding ingredients, also the highest

mean values (3.6 g per day) and upper percentile (14.3 g per day) consumption. French consumers of hazelnuts had a mean intake of 2.2 g per day.

Table 18: Consumption figures of hazelnuts taken from food surveys of the Member States and GEMS/Food Consumption Cluster Diets database for adult population.

Country	All population				Consumers only				Remark
	N	Mean (g/day)	95th %	97.5th %	%	Mean (g/day)	95th %	97.5th %	
GEMS/ Food Cluster B		2.1							ingredients included
GEMS/ Food Cluster E		1.3							
GEMS/ Food Cluster F		0.3							
Spain	1,060	0.1	2.2	2.6	2.0	7.2	15.7	17.3	ingredients not included
Germany	4,030	1.4	8.0	-	37.6	3.6	14.3	-	ingredients included
Ireland	1,379	0.2	-	-	9.9	2.0	-	12.3	ingredients included
France	1,474	0.4	2.2	-	17.8	2.2	7.1	-	ingredients included
United Kingdom	1,724	0.04	-	-	0.6	6.2	-	(max 30.7)	ingredients not included
United Kingdom	1,724	0.2	-	-	15.8	1.3	-	5.4	ingredients included

For pistachios (Table 19) most of the data are affected by the relative rare frequency of their consumption. Thus, high values at the upper percentiles would tend to overestimate the real situation. Estimates from the Spanish diet are far above those from other countries with a similar percentage of consumers. This could be an indication that the Spanish population is a high consumer of pistachios compared to other European countries.

As expected in all tables, the mean values derived from the GEMS/Food Consumption Cluster Diets database for all populations are higher than the population mean values from food surveys for the respective countries. Therefore, it can be concluded that the estimates of the GEMS/Food Consumption Cluster Diets database do not underestimate the consumption values of nuts and can be used to generalise the population exposure results to non-reporting European countries.

Table 19: Consumption figures of pistachios taken from food surveys of the Member States and GEMS/Food Consumption Cluster Diets database for adult population.

Country	All population				Consumers only				Remark
	N	Mean (g/day)	95th %	97.5th %	%	Mean (g/day)	95th %	97.5th %	
GEMS/ Food Cluster B		0.7							ingredients included
GEMS/ Food Cluster E		0.3							
GEMS/ Food Cluster F		<0.1							
Spain	1,060	0.3	5.8	6.9	1.7	19.9	47.5	52.8	ingredients not included
Germany	4,030	0.2	0.2	-	11.4	1.4	7.5	-	ingredients included
Ireland	1,379	<0.1	-	-	0.3	2.8	-	5.7	ingredients included
France	1,474	0.1	0.0	-	3.5	2.7	7.2	-	ingredients included
United Kingdom	1,724	0.07	-	-	0.7	9.3	-	(max 35.7)	ingredients not included
United Kingdom	1,724	0.07	-	-	1.1	6.3	-	25.9	ingredients included

For consumers of the three nut products, the proportion of the population that consumes almonds (2% to 32%) was greater than for hazelnuts (0.6%-37.6%) in several countries. The lowest proportions of consumers were reported for pistachios (0.3%-11.4%). Germany was an exception since there were more consumers of hazelnuts (37.6%) than for almonds (29%). The data analysis has also been performed excluding ingredients and values for the mean intake for consumers only range from 0.8 g/day to 1.6 g/day for almonds, from 1.3 g/day to 3.6 g/day for hazelnuts and from 1.4 g/day to 6.3 g/day for pistachios.

Children

Consumption data for children are presented in Tables 20 to 22. Herein, the different survey methodologies used influenced the results. For example the high percentage of consumers in the 2006 UK food frequency questionnaire were influenced by the time period covered by the survey questions, since food frequency questionnaires normally cover longer time periods than food records. In surveys based on food records the number of survey days is limited and therefore so is the estimate of the percentage of consumers. Not consuming a food in one of the survey days is of course not equal to never consuming the food. However, as the data in Tables 20 to 22 demonstrate, consumption frequency has not affected the mean estimates of the foods consumed.

Based on the consumption data provided by Member States the proportion of children eating almonds (food ingredients included) ranged from 2.9-46.9%. There was a wider range for hazelnuts (1.9% to 81.7%) and a more narrow range for pistachios (0.4% to 2.7%). For hazelnuts higher percentage of consumers was reported for children from Germany (81.7%) and France (47.3%) compared to children from Ireland and Spain. Excluding the values with very low numbers of consumers for all three types of nuts, the mean daily amounts of almonds consumed by children were between 0.9 to 1.3 g/day. The span of hazelnut consumption was also small (0.7 g/day – 1.7 g/day) between the reporting countries. The slightly wider mean consumption of pistachios (0.3 g/day – 4.8 g/day) between countries could be due to the generally low numbers of consumers.

Table 20: Consumption figures of almonds taken from food surveys of the Member States for children.

Country	All population				Consumers only				Remark
	N	Mean (g/day)	95th %	97.5th %	%	Mean (g/day)	95th %.	97.5th %	
Spain	903	<0.1	1.1	1.3	0.2	12.5	23.0	25.0	unshelled, ingredients not included
Spain	903	0.2	2.5	3.0	1.6	9.8	20.8	22.9	shelled, ingredient not included
Germany	475	0.5	2.6	-	46.9	1.0	3.3	-	ingredients included
Ireland	594	<0.1	<0.1	0.2	2.9	1.1	3.8	5.5	ingredients included
France	1,018	0.2	1.2	-	21.2	0.9	-	-	ingredients included
United Kingdom (4-18y)	1,701	<0.1	-	-	0.4	2.1	-	-	ingredients not included
United Kingdom (4-18y)	1,701	0.4	-	-	33.0	1.3	-	4.8	ingredients included
United Kingdom (1.5-4.5y)	1,675	0	-	-	0.2	1.4	-	-	ingredients not included
United Kingdom (1.5-4.5y)	1,675	0.1	-	-	12.0	0.9	-	3.3	ingredients included
United Kingdom	225	0.2	-	-	92.9	0.2	-	-	ingredients not included

Mean consumption values for children and adults are similar. Therefore, children are likely to have higher exposures from almonds, hazelnuts and pistachios expressed on a body weight basis.

From the UK data the analysis shows that including ingredients in the calculation of consumption figures is followed by an increase in population mean consumption figures as well as the number of consumers, and a decrease in the mean amount for consumers only. For exposure calculations only the most recent UK survey data based on the food

frequency questionnaire (FFQ) from 2006 was used, since values of other surveys are within the range of other submitted consumption data.

Table 21: Consumption figures of hazelnuts taken from food surveys of the Member States for children.

Country	All population				Consumers only				Remark
	N	Mean (g/day)	95th %	97.5th %	%	Mean (g/day)	95th %	97.5th %	
Spain	903	<0.1	1.1	1.3	0.4	8.8	17.6	19.3	ingredient not included
Germany	475	1.3	5.0	-	81.7	1.6	5.2	-	ingredients included
Ireland	594	<0.1	<0.1	<0.1	1.9	1.0	2.2	2.9	ingredients included
France	1,018	0.8	3.4	-	47.3	1.7	-	-	ingredients included
United Kingdom (4-18y)	1,701	0.0	-	-	0.1	4.3	-	-	ingredients not included
United Kingdom (4-18y)	1,701	0.2	-	-	21.0	0.9	-	3.8	ingredients included
United Kingdom (1.5-4.5y)	1,675	0.0	-	-	0.2	1.0	-	-	ingredients not included
United Kingdom (1.5-4.5y)	1,675	<0.1	-	-	11.0	0.7	-	2.7	ingredients included
United Kingdom	225	0.2	-	-	96.9	0.2	-	-	ingredients not included

Table 22: Consumption figures of pistachios taken from food surveys of the Member States for children.

Country	All population				Consumers only				Remark
	N	Mean (g/day)	95th %	97.5th %	%	Mean (g/day)	95th %	97.5th %	
Spain	903	0.1	1.3	1.5	0.6	9.4	16.5	17.9	ingredients not included
Germany	475	< 0.1	<0.1	-	1.9	0.3	1.1	-	ingredients included
Ireland	594	<0.1	<0.1	<0.1	0.7	4.8	7.9	7.9	ingredients included
France	1,018	0.1	<0.1	-	2.7	3.8	-	-	ingredients included
United Kingdom (4-18y)	1,701	0.01	-	-	0.3	3.4	-	-	ingredients not included
United Kingdom (4-18y)	1,701	0.0	-	-	0.5	2.6	-	-	ingredients included
United Kingdom (1.5-4.5y)	1,675	<0.1	-	-	0.2	4.3	-	-	ingredients not included
United Kingdom (1.5-4.5y)	1,675	<0.1	-	-	0.4	3.5	-	-	ingredients included
United Kingdom	225	0.5	-	-	93.3	0.6	-	-	ingredients not included

Some countries only reported values at an aggregated level, such as "total nuts", or data that do not come from food surveys but from other data sources. These values are presented in Table 23. Respective values from the GEMS/Food Consumption Cluster Diets database have also been included.

Table 23: Data only available as aggregated food groups (ingredients included).

Food group	GEMS/ Food (mean of all population in g/day)			Adults		Children
	Cluster B	Cluster E	Cluster F	Estonia (ingredients not included)	Hungary (nut-like products and oilseed)	Sweden
Treenuts	21.5	5.5	10.2	0.5	-	0.5
Oilseeds (including groundnuts)	65.2	62.1	39.4	-	-	-
Total nuts and oilseeds	91.9	69.2	51.3	-	5.5	-

Vegans and Vegetarians

Special groups of interest are vegans and vegetarians because their consumption of nuts is likely to be higher than non vegetarians to boost their protein intake. Two countries (UK and France) provided consumption data for those two groups.

The data from the British survey are given in Table 24. The mean almond consumption for the British vegetarians was 4.8 g/day. This was not duplicated for hazelnuts and pistachios.

Table 24: Consumption figures for British vegans and vegetarians (ingredients not included) in gram per day.

	%	Mean of all population	Mean of consumers	Highest Value
Peanuts	94.8	3.8	4.1	150.0
Hazelnuts	70.4	1.8	2.6	29.9
Almonds	92.2	4.4	4.8	178.1
Pistachios	91.9	1.0	1.1	28.5
Cashews	91.5	6.8	7.4	150.0
Brazil nuts	94.1	5.1	5.4	60.0
Walnuts	90.7	2.6	2.8	59.0
Pecans	91.1	0.5	0.5	60.0
Chestnuts	90.0	0.4	0.4	26.3
Macadamia	95.2	0.4	0.5	28.5
Pine nuts	93.3	1.7	1.8	25.0
Mixed nuts	79.3	2.3	2.9	119.7

Values presented in Table 25 regarding the French population can only be compared with the data of the GEMS/Food Consumption Cluster Diets database in Table 16, because of the aggregated food categories. There is no further indication that vegans and vegetarians would eat more nuts than the rest of the population.

Table 25: Consumption figures for the sum of all nuts and oilseeds of French vegans and vegetarians in gram per day (ingredients not included).

	N	Mean	95th %
Lacto-ovo-vegetarian	74	32.4	101.4
Lacto-vegetarian	38	21.7	83.6
Vegan/Macrobiotic	26	43.3	174.0

Data used in exposure calculations

It can be concluded that enough information is available to obtain a good picture of the variation in nut consumption between countries in the EU. Exposure calculations for children and adults have been performed using the population mean of food surveys

conducted by Member States and the GEMS/Food Consumption Cluster Diets database was used to calculate mean exposure scenarios and the exposure to other food items. To estimate high level dietary exposure to aflatoxins the 95th percentile values (or 97.5th percentile if not available) of the nut consumption data based on food surveys conducted by Member States were used.

For the estimates of vegetarian consumption the exposure from other foods is taken from the GEMS/Food Consumption Cluster Diets database and the values for nut consumption are taken from the UK survey for vegans and vegetarians.

3.6 Assessment of the exposure

Scenarios

For almost all of these data, the values for AFM1 concentration were below the limit of detection or very low in comparison to AFB1, taking into account the difference in potency, and therefore AFM1 was not included in the assessments of total dietary exposure.

Intakes from almonds, hazelnuts and pistachios have been assessed by the use of aggregated data from the GEMS/Food Consumption Cluster Diets database or national survey information at an individual level. Data from the GEMS/Food Consumption Cluster Diets database enabled extrapolation to other non-reporting Member States, whilst national survey information allowed a more accurate assessment and identification of groups of high level consumers. The exposure from food sources other than the three nuts could only be calculated by using the GEMS/Food Consumption Cluster Diets database because not all Member States provided data for all food groups of interest. Data for consumption and occurrence are described in more detail in the previous chapter.

Body weights were not available for all countries, so the Panel decided to use 60 kg body weight for adults and 15 kg for children as standard values.

All data were combined in four scenarios for the exposure assessment. In general, a scenario is characterised by the following decisions:

- Consumption
  a) taking into account "all population" or "consumers only";
  b) mean or high percentile of a);
  c) GEMS/Food Consumption Cluster Diets data or data from individual surveys;
  d) definition of exposure from other food sources;
  e) selection of a subgroup.

- Occurrence data
  f) AFB1 or total aflatoxins;
  g) upper bound or lower bound estimates to handle values below the LOD;
  h) mean or high percentiles for f);
  i) different cut off points to simulate the MLs.

It was not feasible to present results of all possible combinations for all Member States and subgroups. Therefore, the following four scenarios were explored:

- **Scenario 1 - average exposure -**
  a) "all population" for hazelnuts, almonds and pistachios;
  b) population mean consumption data for all three nuts;
  c) GEMS/Food Consumption Cluster Diets data and data from individual surveys; exposure from other food sources from GEMS/Food Consumption Cluster Diets database mean for all population and mean of the aflatoxin occurrence data;
  d) adults, children and vegetarians;
  e) total aflatoxins;
  f) upper bound and lower bound estimates;
  g) mean values for occurrence data;
  h) cut off points of 4, 8, and 10 μg/kg to simulate different proposed MLs.

- **Scenario 2 - high level exposure for almonds -**
  a) taking into account "all population" for hazelnuts and pistachios and "consumers only" for almonds;
  b) mean for hazelnuts and pistachios, but high level for almond consumption
  c) data from individual surveys;
  d) same as Scenario 1;
  e) adults;
  f) total aflatoxins;
  g to i) same as Scenario 1.

- **Scenario 3 - high level exposure for hazelnuts -**
  a) taking into account "all population" for almonds and pistachios and "consumers only" for hazelnuts;
  b) mean for almonds and pistachios, but high level for hazelnut consumption;
  c) same as Scenario 2;
  d) same as Scenario 1;
  e) same as Scenario 2;
  f) total aflatoxins;
  g to i) same as Scenario 1.

- **Scenario 4 - high level exposure for pistachios -**
  a) taking into account "all population" for almonds and hazelnuts and "consumers only" for pistachios;
  b) mean for almonds and hazelnuts, but high level of pistachios consumption;
  c) same as Scenario 2;
  d) same as Scenario 1;
  e) same as Scenario 2;
  f) total aflatoxins;
  g to i) same as Scenario 1.

These scenarios involve a number of assumptions. Firstly, mean levels of aflatoxin contamination were assumed to be of most relevance for long-term exposures. The Panel considered whether high level occurrence data should be used, but found no evidence that particular sources of nuts were consistently highly contaminated and therefore brand preference would not affect average long-term exposure.

To estimate exposure under the current legislation, occurrence data with levels below or at the MLs were used assuming that all other foods were detected by food surveillance and prevented from reaching the market and therefore not consumed. This assumption does not reflect the true situation, but since there is no information on which to base assumptions of the effects of surveillance systems before and after any change to the permitted MLs, this assumption provides the best basis for a comparison of the current situation with hypothetical future scenarios.

Table 26 illustrates Scenario 1 using mean occurrence data and mean consumption data based on the current situation for adults. These data are used in chapter 3.7 when calculating the potential impact of increasing the maximum levels for aflatoxins. All values were truncated at 4 µg/kg and the mean estimates of exposure to total aflatoxins in the European Member States ranged between 0.35 and 0.84 ng/kg b.w. per day for lower bound and between 0.69 and 1.93 ng/kg b.w. per day for upper bound. Northern countries clustered in Group F had the lowest intake estimates and southern European countries the highest, because of the exposure from other foods. The contribution of the three nut products to overall exposure varied between 0.3 to 2.3% for the first scenario.

From Table 26 it can also be seen that the contribution of exposure from other foods differed in the three GEMS/Food regions. The fractions of exposure from other nuts and spices were low in all regions. In the southern countries the exposure from maize and in northern countries exposure from oilseeds were the most important exposure sources for exposure from foods other than the three nuts. These results are illustrated in Figure 12.

It is likely that the exposure from maize for Cluster B (and hence for Spain) is an over-estimate because of the inclusion of maize oil, which is expected to contain lower

concentrations of aflatoxins than other maize products. No data were available regarding the relative proportions of maize oil and other maize products in the consumption data to allow a more accurate assessment. The Panel analysed the impact of this over-estimation by assuming that the consumption of maize might have been 10 times lower. This calculation resulted in lower bound to upper bound ranges of 0.57 – 1.1 ng/kg b.w. per day for Cluster B and 0.55 – 1.07 ng/kg b.w. per day for Spain, which are similar to the estimates from other countries.

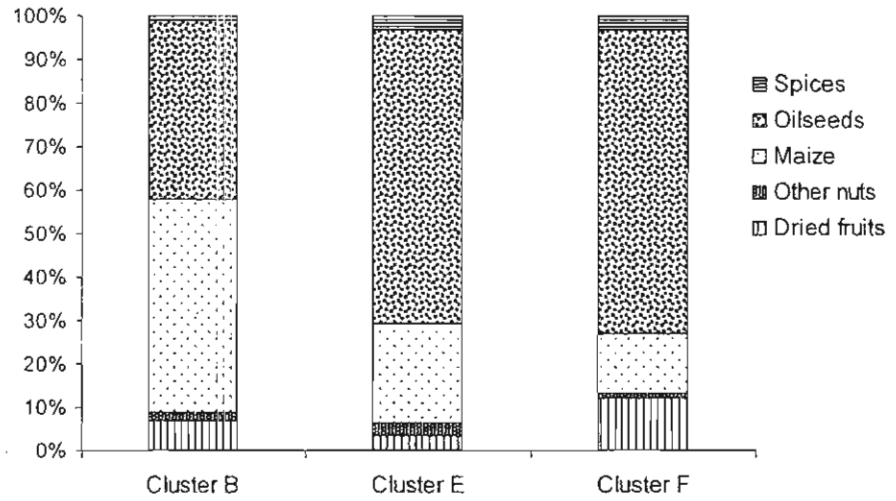

Figure 12: Percentage contribution of other foods to total exposure based on GEMS/Food consumption data and collected occurrence data from Member States as given in Table 26.

Table 26: Scenario 1 "average exposure" to total aflatoxins in ng/kg b.w. per day truncating occurrence data at its current EU MLs for adults.

Food	Values below LOD	Cluster B	Cluster E	Cluster F	Spain	Germany	Ireland	France	UK
Almonds	lower bound	0.006	0.003	0.002	0.002	0.001	0.000	0.002	0.002
	upper bound	0.013	0.007	0.005	0.003	0.003	0.001	0.003	0.003
Hazelnuts	lower bound	0.011	0.007	0.002	0.001	0.007	0.001	0.002	0.001
	upper bound	0.019	0.011	0.003	0.001	0.012	0.002	0.004	0.002
Pistachios	lower bound	0.002	0.001	0.000	0.001	0.001	0.000	0.000	0.000
	upper bound	0.005	0.002	0.001	0.002	0.001	0.001	0.001	0.001
Other food items	lower bound	0.819	0.546	0.348	0.819		0.546		
	upper bound	1.898	1.077	0.678	1.898		1.077		
- Other nuts	lower bound	0.014	0.012	0.004	0.014		0.012		
	upper bound	0.042	0.034	0.010	0.042		0.034		
- Maize	lower bound	0.301	0.080	0.030	0.301		0.080		
	upper bound	0.929	0.246	0.091	0.929		0.246		
- Oilseeds	lower bound	0.445	0.416	0.272	0.445		0.416		
	upper bound	0.776	0.726	0.475	0.776		0.726		
- Dried fruits	lower bound	0.042	0.012	0.026	0.042		0.012		
	upper bound	0.130	0.037	0.081	0.130		0.037		
- Spices	lower bound	0.016	0.027	0.016	0.016		0.027		
	upper bound	0.021	0.034	0.021	0.021		0.034		
Total	**lower bound**	**0.838**	**0.557**	**0.352**	**0.822**	**0.556**	**0.548**	**0.550**	**0.549**
	upper bound	**1.934**	**1.097**	**0.687**	**1.904**	**1.094**	**1.080**	**1.085**	**1.083**

Figure 13 shows that the different patterns of consumption in the Member States affect the relative importance of the exposure to aflatoxins from each nut type. In all countries except Spain, exposure from pistachios was the lowest. In Spain hazelnuts gave the lowest and almonds the highest exposure. Almonds were also most important in UK and the Northern European countries of Cluster F. In all other cases hazelnuts gave the highest aflatoxin exposure of the three types of nuts.

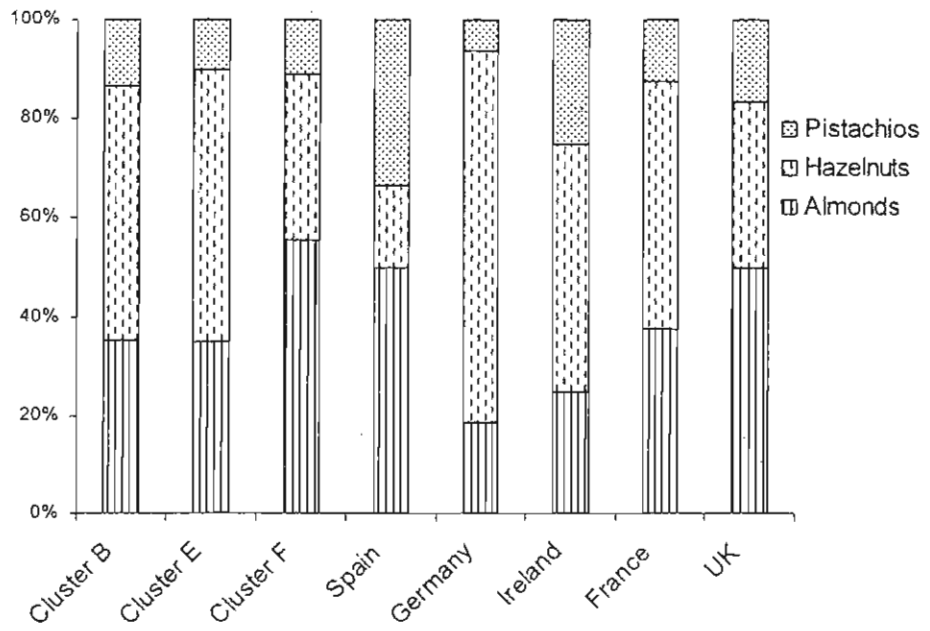

Figure 13: Percentages of almonds, hazelnuts and pistachios of the total exposure to aflatoxins from all three nuts based on Scenario 1 as given in Table 26.

The exposure estimates for the "high level exposure"scenarios 2, 3 and 4 under the current situation are presented in Table 27. The highest effect could be seen for high pistachio consumers in scenario 4. There the maximum total exposure increases from the scenario 1 population average of 0.8-1.9 ng/kg b.w. per day (lower bound to upper bound) up to 1.0-2.3 ng/kg b.w. per day in Spain. For high consumers of hazelnuts the values were 0.9-2.0 ng/kg b.w. per day. In Germany and Ireland high hazelnut consumers gave the highest increase in total exposure. The percentage increases in scenarios 2, 3 and 4 compared with scenario 1 are shown in figure 14.

Table 27: Scenario 2-4 "high level exposure" to total aflatoxins in ng/kg b.w. per day truncating occurrence data at MLs of the current European legislation for adults.

Food	Values below LOD	Spain	Germany	Ireland	France	UK
Scenario 2 "high level exposure almonds"						
Almonds	lower bound	0.066	0.016	0.018	0.011	0.025
	upper bound	0.146	0.036	0.039	0.025	0.055
Hazelnuts	*lower bound*	*0.001*	*0.007*	*0.001*	*0.002*	*0.001*
	upper bound	*0.001*	*0.012*	*0.002*	*0.004*	*0.002*
Pistachios	*lower bound*	*0.001*	*0.001*	*0.000*	*0.000*	*0.000*
	upper bound	*0.002*	*0.001*	*0.001*	*0.001*	*0.001*
Other food items	*lower bound*	*0.819*		*0.546*		
	upper bound	*1.898*		*1.077*		
Total	**lower bound**	**0.887**	**0.571**	**0.566**	**0.560**	**0.573**
	upper bound	**2.047**	**1.127**	**1.119**	**1.106**	**1.135**
Scenario 2 "high level exposure hazelnuts"						
Almonds	*lower bound*	*0.002*	*0.001*	*0.000*	*0.002*	*0.002*
	upper bound	*0.003*	*0.003*	*0.001*	*0.003*	*0.003*
Hazelnuts	lower bound	0.081	0.074	0.064	0.037	0.028
	upper bound	0.139	0.126	0.109	0.063	0.048
Pistachios	*lower bound*	*0.001*	*0.001*	*0.000*	*0.000*	*0.000*
	upper bound	*0.002*	*0.001*	*0.001*	*0.001*	*0.001*
Other food items	*lower bound*	*0.819*		*0.546*		
	upper bound	*1.898*		*1.077*		
Total	**lower bound**	**0.903**	**0.622**	**0.611**	**0.585**	**0.576**
	upper bound	**2.042**	**1.207**	**1.187**	**1.144**	**1.129**
Scenario 4 "high level exposure pistachios"						
Almonds	*lower bound*	*0.002*	*0.001*	*0.000*	*0.002*	*0.002*
	upper bound	*0.003*	*0.003*	*0.001*	*0.003*	*0.003*
Hazelnuts	*lower bound*	*0.001*	*0.007*	*0.001*	*0.002*	*0.001*
	upper bound	*0.001*	*0.012*	*0.002*	*0.004*	*0.002*
Pistachios	lower bound	0.158	0.025	0.019	0.024	0.086
	upper bound	0.348	0.055	0.042	0.053	0.190
Other food items	*lower bound*	*0.819*		*0.546*		
	upper bound	*1.898*		*1.077*		
Total	**lower bound**	**0.980**	**0.580**	**0.567**	**0.574**	**0.635**
	upper bound	**2.251**	**1.147**	**1.121**	**1.137**	**1.272**

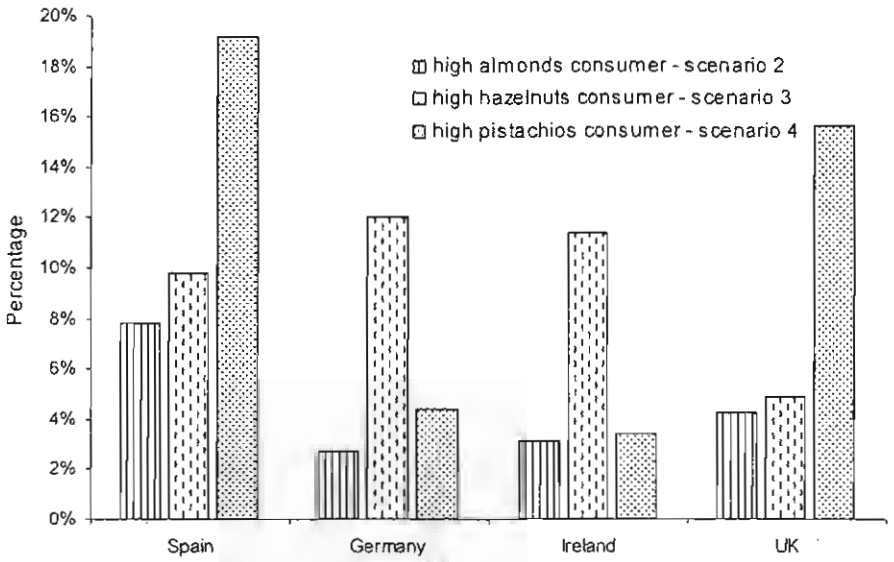

Figure 14: Increase in total exposure to aflatoxins for upper bound values assuming high consumption of almonds, hazelnuts or pistachios respectively (scenarios 2, 3 and 4) compared with average exposure of scenario 1.

Population sub-groups

The question as to whether vegans and vegetarians have a higher exposure to aflatoxins than the rest of the population due to higher nut intake to boost their protein intake has been explored. Data were available from a FFQ performed in 2006 in the United Kingdom, supporting the Commission in assessing nut consumption for vegans and vegetarians.

Further, it is important to determine the exposure for children, who are often more exposed to contaminants because of their lower body weight. In this opinion a body weight of 15 kg is assumed for children. The data for food consumption were taken from National Food Surveys as described in section 3.5.

Dietary exposures were calculated for all datasets as presented in Table 28 together with the highest and lowest exposure value from scenario 1 for the adult population. These results demonstrate that all estimates are lower than those for the average population scenario in Table 26.

The estimated fraction of nut exposure from the whole diet was also higher for the FFQ data from 2006. For total aflatoxins it was 2.4% for the upper bound estimate instead of 1.9% for the maximum of the adult estimates based on national survey data. However, the exposure from other food items was not adjusted explicitly for vegetarians and children, who might have different eating patterns for foods like dried fruit and maize which could affect the total dietary exposure to aflatoxins.

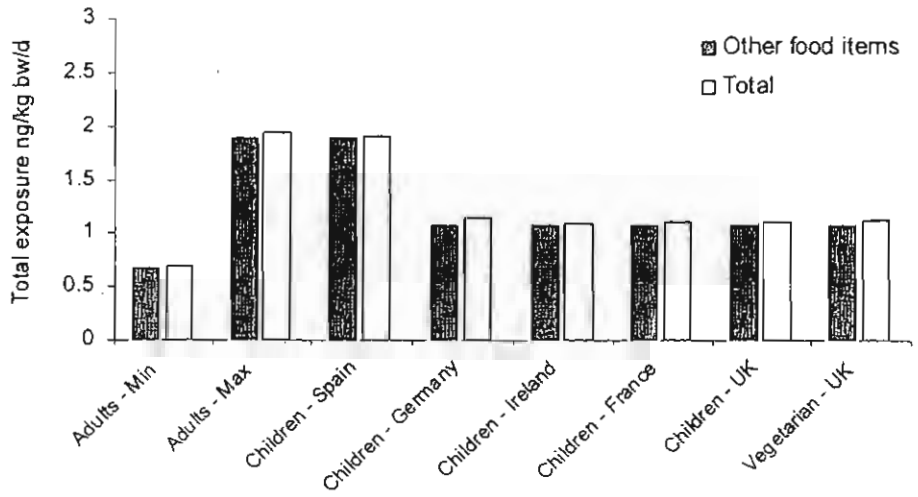

Figure 15: Total dietary aflatoxin exposure and aflatoxin exposure from food items other than almonds, hazelnuts and pistachios: upper bound values for minimum and maximum scenario 1 estimates for adults compared to exposure for children and vegetarians.

Figure 15 and Table 28 illustrate that the exposure from other food items for Spanish children is the same as that for adults. This pattern is due to the fact that the exposure from other food items based on Cluster B was used for children and for adults.

The aflatoxin exposure from the three nuts without taking into account exposure from other food items is shown in Figure 16. The consumption patterns of children and of UK vegetarians differed from those of the general adult population. Hence, almond and pistachio consumption was higher in vegans and vegetarians compared to the maximum consumption of adults. Hazelnut and pistachio consumption of children from Germany, France and the UK respectively were also higher than the maximum value for adults.

Table 28: Scenario 1 "average exposure" to total aflatoxins in ng/kg b.w. per day truncating occurrence data at MLs of the current European legislation - children and vegetarian in comparison to minimum and maximum adult exposure values (from Table 24).

Food	Values below LOD	Adults		Children					Vegetarian
		Min	Max	Spain	Germany	Ireland	France	UK	UK
Almonds	lower bound	0.000	0.006	0.002	0.006	0.001	0.002	0.002	0.013
	upper bound	0.001	0.013	0.005	0.013	0.003	0.005	0.005	0.029
Hazelnuts	lower bound	0.001	0.011	0.002	0.027	0.002	0.017	0.004	0.009
	upper bound	0.001	0.019	0.004	0.046	0.004	0.028	0.007	0.016
Pistachios	lower bound	0.000	0.002	0.001	0.001	0.001	0.001	0.007	0.003
	upper bound	0.001	0.005	0.003	0.003	0.003	0.003	0.015	0.007
Other foods	lower bound	0.348	0.819	0.819		0.546			0.546
	upper bound	0.678	1.898	1.898		1.077			1.077
- Other nuts	lower bound	0.004	0.014	0.014		0.012			0.012
	upper bound	0.010	0.042	0.042		0.034			0.034
- Maize	lower bound	0.030	0.301	0.301		0.080			0.080
	upper bound	0.091	0.929	0.929		0.246			0.246
- Oilseeds	lower bound	0.272	0.445	0.445		0.416			0.416
	upper bound	0.475	0.776	0.776		0.726			0.726
- Dried fruits	lower bound	0.012	0.042	0.042		0.012			0.012
	upper bound	0.037	0.130	0.130		0.037			0.037
- Spices	lower bound	0.016	0.027	0.016		0.027			0.027
	upper bound	0.021	0.034	0.021		0.034			0.034
Total	lower bound	0.352	0.838	0.825	0.592	0.563	0.578	0.571	0.572
	upper bound	0.687	1.934	1.910	1.139	1.086	1.114	1.104	1.310

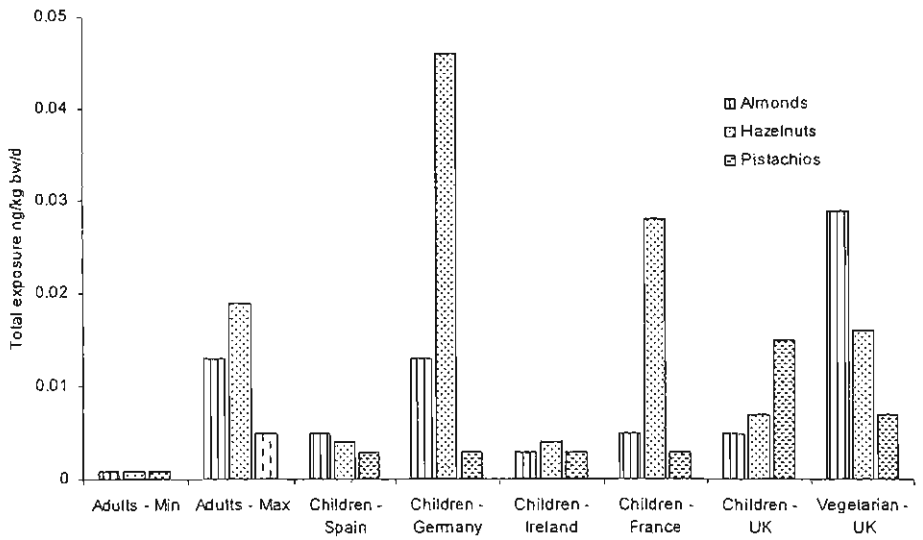

Figure 16: Aflatoxin exposure from almonds, hazelnuts and pistachios for children and vegans/vegetarians compared to adults for upper bound values of scenario 1.

Influence of setting the LOD

In tables 26 to 28, two standard approaches were used in dealing with the LOD-values. Use of the upper bound gives an overestimation of the occurrence levels and therefore of exposure, whereas lower bound gives an underestimation. Actual exposure will be within this range of values and as it is illustrated in Figure 17, changes in the handling of values below the LOD have a relatively high impact. This can be explained by the high numbers of values below the LOD in the data used for estimation of intake from other foods other than almonds, hazelnuts and pistachios.

The over-estimation resulting from the use of the upper bound entails a precautionary approach to assessment of exposure and hence of potential health effects. However, this over-estimation will mask the relative potential impact of increasing the MLs and therefore the lower bound approach is also important.

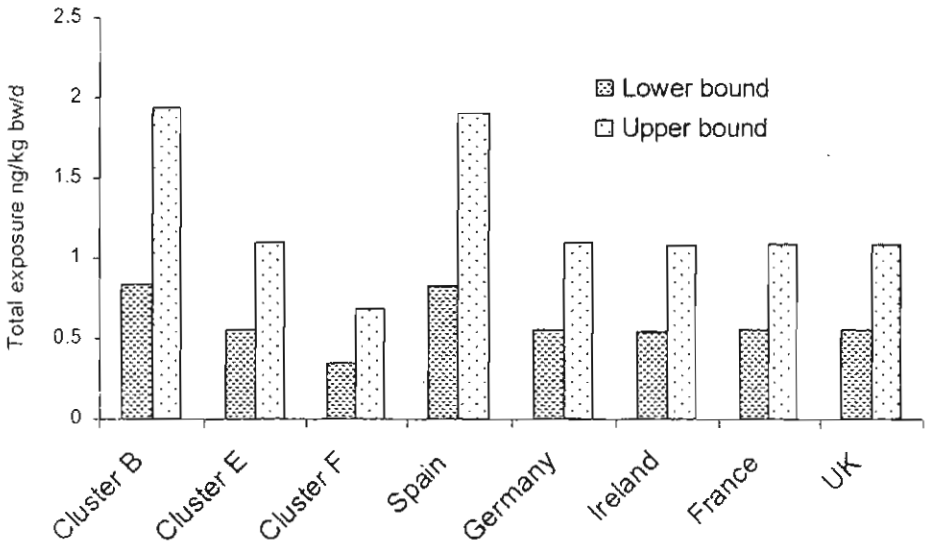

Figure 17: Total exposure to total aflatoxins in ng/kg b.w. per day dependent on different handling of values below LOD/LOQ as given in Table 26.

Conclusions and recommendations

High level consumers of pistachios were calculated to have the highest total dietary exposure to aflatoxins, with an upper bound estimate in the range of 1.1 to 2.3 ng/kg b.w. per day. The ranges of upper bound estimates for high level consumers of almonds were 1.1-2.1 ng/kg b.w. per day and for hazelnuts 1.1-2.0 ng/kg b.w. per day. The highest values were all from Spain with a survey methodology that was not fully appropriate for chronic exposure assessments. The second highest values were in the range of 1.2 to 1.3

ng/kg b.w. per day for all three types of nuts. No unique picture was apparent for children or for vegetarians.

The effect of the handling of values below the LOD is important in assessing the total dietary exposure to aflatoxins and also for calculating the proportion of aflatoxin exposure via nuts in relation to total exposure. Because of the high impact of the LOD on the results, an improvement in the analytical methods to increase the sensitivity and thus lower the LOD would reduce the uncertainty in the exposure assessment. It is recommended to make more of an effort in harmonising analytical methodology and to use more sensitive methods for quantifying aflatoxins in food products if the data are to be used specifically for risk assessment purposes.

Some Member States reported a few results from testing of aflatoxins in other foods (e.g. rice, cacao, tea), but there were not enough samples reported to take these food groups into consideration. The Panel recommends more extensive analyses of aflatoxin levels in other foodstuffs to check that there is no unknown contributor to total dietary aflatoxin exposure.

Furthermore, there is a need for more accurate food consumption data to assess exposure from other food items. A systematic and representative sampling of all suspected food groups is needed based on individual food survey data from Member States to replace the imprecise data from the GEMS/Food Consumption Cluster Diets database. When the EFSA concise food consumption database (under construction at the moment) is available it is recommended that a more precise assessment of total dietary aflatoxin exposure be conducted.

3.7 Potential impact of increasing the regulatory MLs for aflatoxins

To give a clear and understandable picture the information is aggregated to the most important figures for the following Tables. To show the full range of potential impact on exposure the minimum and maximum values from all countries are selected and presented in the Tables.

The effect of changing the MLs of aflatoxins in almonds, hazelnuts and pistachios from 4 to 8 or 10 µg/kg was simulated in further exposure analyses for all four scenarios with constant aflatoxin levels for the exposure from other foods (Tables 29-32).

In Tables 29-32, the consumption figures for the three nuts for the adult population (taken from Tables 17-19) are given in the columns of the 3 tables on the left hand side of the page. In the rows of the left-hand tables for each of the three nuts the mean lower bound and upper bound occurrence levels after truncation at the proposed MLs at 4, 8 and 10 µg/kg (taken from Tables 6-8) are given. All resulting exposure values can be read from the body of the left-hand tables. As an example: the exposure value for hazelnut exposure

in France according to Scenario 1 can be read from Table 29. Increasing the ML from 4 to 10 µg/kg increases the lower bound exposure estimate from 0.002 to 0.004 ng/kg b.w. per day, assuming a body weight of 60 kg. This is the product of 0.4 g per ay hazelnut consumption per day and 0.31 and 0.57 µg/kg as the mean of the hazelnut occurrence data for total aflatoxins truncated at a level of 4 and 10 µg/kg, respectively. The tables on the right give the sum of the lowest and highest total aflatoxin exposure (including and excluding exposure from other foods) in dependence on the hypothetical ML as well as the resulting increase in percent. As an example for Scenario 1: the country/region with minimal exposure is defined by the lower bound estimate of Cluster F. The sum of the values listed for a ML of 8 µg/kg aflatoxin for almonds (0.003 ng/kg b.w. per day), hazelnuts (0.002 ng/kg b.w. per day) and pistachios (0.001 ng/kg b.w. per day) is 0.006 ng/kg b.w. per day (see second row of the upper right table). Adding the value of "exposure from other food items" this figure increases to 0.354 ng/kg b.w. per day (see header "adding other food items"). The percentages in the right-hand table illustrate the increase in exposure compared to truncated occurrence data at a ML of 4 µg/kg aflatoxins. The same calculations are performed for the maximum exposure case (lower right table).

Table 29 shows that increasing the MLs from 4 to 8 or 10 µg/kg would result in an average increase of total dietary aflatoxin exposure for the average adult population in the region of 1%. The impact on high level consumers of nuts could be greater (as shown in Tables 30-32), particularly for high level consumers of hazelnuts or pistachios. The highest estimate for high level adult total aflatoxin exposure of 0.98-2.25 ng/kg b.w. per day (lower bound to upper bound) increased by 6-14% to 1.12-2.39 ng/kg b.w. per day for a ML of 8 µg/kg and by 9-21% to 1.19-2.45 ng/kg b.w. per day for an ML of 10 µg/kg. These estimates relate to high level consumers of pistachios but could be overestimates as the consumption data are based on a small proportion of consumers. As already mentioned the use of upper bound data results in higher estimates of total exposure, but the relative increases are higher for the lower bound data. Actual values for both the total exposure and the relative increase will fall within the range of the lower bound to the upper bound if the occurrence data fall within the specified ranges (i.e. none exceed the respective ML).

The relative importance of the sum of exposure for the three nuts compared to the exposure from other food items differs in the high consumption scenarios and changes with MLs. The lowest impact of nut exposure to overall exposure can be seen for the upper bound estimate in the 4 µg/kg case in scenario 1, where the aflatoxin exposure from nuts was 1.9% of the total aflatoxin exposure. The highest percentage for aflatoxin exposure from nuts compared to the total exposure was 22.5%, in scenario 4 for the upper bound estimate assuming a ML of 10 µg/kg.

Conclusions and recommendations

For the average consumers changing the ML for total aflatoxins in almonds, hazelnuts and pistachios from 4 to 10 ng/kg b.w. per day would result in an increase in total dietary aflatoxin exposure of about 1%.

Population groups with high nut consumption are exposed to higher levels of aflatoxins in all assessments. Changing the MLs for the three nut products could have an impact for some of these groups, with a potential maximum increase of up to 20% (from 0.98 to 1.19 ng/kg b.w. per day) if the MLs were increased from 4 to 10 µg/kg and strictly enforced. If, as is expected, nuts exceeding the MLs are occasionally consumed, the total long term average dietary aflatoxin exposures might be higher, but the impact of raising the regulatory ML would be less.

In the cases of high consumption patterns for one of the three nuts and mean occurrence levels aflatoxin exposure from nuts initially seemed to be low in relation to the aflatoxin exposure from other foods. The proportion of aflatoxin exposure from the three nuts increased in importance in some of the calculated scenarios particularly for some Member States. However, it should be noted that the use of the mean is conservative compared to the median and the mean is more sensitive to changes in the ML.

A summary of the various exposure scenarios can be found in Table 33.

Table 29: Changes in exposure calculations for **scenario 1 "average population"** for adult population by changing the MLs (LB=lower bound estimate, UB= upper bound estimate, consumption values are given as g/day, occurrence data and MLs as µg/kg, exposure values as ng/kg b.w. per day).

Almonds

			Consumption								
			Cl. B	Cl. E	Cl. F	Spain	Germany	Ireland	France	UK	
ML	Occurrence		1.9	1.0	0.8	0.5	0.4	0.1	0.5	0.5	
4	LB	0.18	0.006	0.003	0.002	0.002	0.001	0.000	0.002	0.002	E
	UB	0.40	0.013	0.007	0.005	0.003	0.003	0.001	0.003	0.003	x
											p
8	LB	0.24	0.008	0.004	0.003	0.002	0.002	0.000	0.002	0.002	o
	UB	0.46	0.015	0.008	0.006	0.004	0.003	0.001	0.004	0.004	s
											u
10	LB	0.29	0.009	0.005	0.004	0.002	0.002	0.000	0.002	0.002	r
	UB	0.50	0.016	0.008	0.007	0.004	0.003	0.001	0.004	0.004	e

Hazelnuts

			Consumption								
			Cl. B	Cl. E	Cl. F	Spain	Germany	Ireland	France	UK	
ML	Occurrence		2.1	1.3	0.3	0.1	1.4	0.2	0.4	0.2	
4	LB	0.31	0.011	0.007	0.002	0.001	0.007	0.001	0.002	0.001	E
	UB	0.53	0.019	0.011	0.003	0.001	0.012	0.002	0.004	0.002	x
											p
8	LB	0.46	0.016	0.01	0.002	0.001	0.011	0.002	0.003	0.002	o
	UB	0.68	0.024	0.015	0.003	0.001	0.016	0.002	0.005	0.002	s
											u
10	LB	0.57	0.02	0.012	0.003	0.001	0.013	0.002	0.004	0.002	r
	UB	0.78	0.027	0.017	0.004	0.001	0.018	0.003	0.005	0.003	e

Pistachios

			Consumption								
			Cl. B	Cl. E	Cl. F	Spain	Germany	Ireland	France	UK	
ML	Occurrence		0.7	0.3	0.1	0.3	0.2	0.1	0.1	0.1	
4	LB	0.20	0.002	0.001	0.000	0.001	0.001	0.000	0.000	0.000	E
	UB	0.44	0.005	0.002	0.001	0.002	0.001	0.001	0.001	0.001	x
											p
8	LB	0.37	0.004	0.002	0.001	0.002	0.001	0.001	0.001	0.001	o
	UB	0.61	0.007	0.003	0.001	0.003	0.002	0.001	0.001	0.001	s
											u
10	LB	0.46	0.005	0.002	0.001	0.002	0.002	0.001	0.001	0.001	r
	UB	0.69	0.008	0.003	0.001	0.003	0.002	0.001	0.001	0.001	e

Sum for minimum of all countries/ regions (Cluster F)

	ignoring other foods		adding other foods	
ML	exposure	increase	exposure	increase
4	0.004	-	0.352	-
8	0.006	43%	0.354	0.5%
10	0.008	75%	0.356	0.9%

Sum for maximum of all countries/ regions (Cluster B)

	ignoring other foods		adding other foods	
ML	exposure	increase	exposure	increase
4	0.037	-	1.934	-
8	0.046	25%	1.944	0.5%
10	0.051	41%	1.949	0.8%

Table 30: Changes in exposure calculations for **scenario 2 "high level exposure for almonds"** for adult population by changing the MLs (LB=lower bound estimate, UB= upper bound estimate, consumption vales are given as g/d, occurrence data and MLs as µg/kg, exposure values as ng/kg b.w. per day).

Almonds			Consumption					
			Spain	Germany	Ireland	France	UK	
ML	Occurrence		21.9	5.4	5.9	3.7	8.3	
4	LB	0.18	0.066	0.016	0.018	0.011	0.025	E
	UB	0.40	0.146	0.036	0.039	0.025	0.055	x
								p
8	LB	0.24	0.088	0.022	0.024	0.015	0.033	o
	UB	0.46	0.168	0.041	0.045	0.028	0.064	s
								u
10	LB	0.29	0.106	0.026	0.029	0.018	0.040	r
	UB	0.50	0.183	0.045	0.049	0.031	0.069	e

Hazelnuts			Consumption					
			Spain	Germany	Ireland	France	UK	
ML	Occurrence		0.1	1.4	0.2	0.4	0.2	
4	LB	0.31	0.001	0.007	0.001	0.002	0.001	E
	UB	0.53	0.001	0.012	0.002	0.004	0.002	x
								p
8	LB	0.46	0.001	0.011	0.002	0.003	0.002	o
	UB	0.68	0.001	0.016	0.002	0.005	0.002	s
								u
10	LB	0.57	0.001	0.013	0.002	0.004	0.002	r
	UB	0.78	0.001	0.018	0.003	0.005	0.003	e

Pistachios			Consumption					
			Spain	Germany	Ireland	France	UK	
ML	Occurrence		0.3	0.2	0.1	0.1	0.1	
4	LB	0.20	0.001	0.001	0.000	0.000	0.000	E
	UB	0.44	0.002	0.001	0.001	0.001	0.001	x
								p
8	LB	0.37	0.002	0.001	0.001	0.001	0.001	o
	UB	0.61	0.003	0.002	0.001	0.001	0.001	s
								u
10	LB	0.46	0.002	0.002	0.001	0.001	0.001	r
	UB	0.69	0.003	0.002	0.001	0.001	0.001	e

Sum for minimum of all countries/ regions (Ireland)

	ignoring other foods		adding other foods	
ML	exposure	increase	exposure	increase
4	0.019	-	0.566	-
8	0.026	35%	0.572	1.18%
10	0.031	64%	0.578	2.14%

Sum for maximum of all countries/ regions (Spain)

	ignoring other foods		adding other foods	
ML	exposure	increase	exposure	increase
4	0.149	-	2.047	-
8	0.172	15%	2.070	1.12%
10	0.187	26%	2.085	1.86%

Table 31: Changes in exposure calculations for **scenario 3 "high level exposure for hazelnuts"** for adult population by changing the MLs (LB=lower bound estimate, UB= upper bound estimate, consumption values are given as g/d, occurrence data and MLs as µg/kg, exposure values as ng/kg b.w. per day).

Almonds

			Consumption					
			Spain	Germany	Ireland	France	UK	
ML	Occurrence		0.5	0.4	0.1	0.5	0.5	
4	LB	0.18	0.002	0.001	0.000	0.002	0.002	E
	UB	0.40	0.003	0.003	0.001	0.003	0.003	x
								p
8	LB	0.24	0.002	0.002	0.000	0.002	0.002	o
	UB	0.46	0.004	0.003	0.001	0.004	0.004	s
								u
10	LB	0.29	0.002	0.002	0.000	0.002	0.002	r
	UB	0.50	0.004	0.003	0.001	0.004	0.004	e

Hazelnuts

			Consumption					
			Spain	Germany	Ireland	France	UK	
ML	Occurrence		15.7	14.3	12.3	7.1	5.4	
4	LB	0.31	0.081	0.074	0.064	0.037	0.028	E
	UB	0.53	0.139	0.126	0.109	0.063	0.048	x
								p
8	LB	0.46	0.120	0.110	0.094	0.054	0.041	o
	UB	0.68	0.178	0.162	0.139	0.080	0.061	s
								u
10	LB	0.57	0.149	0.136	0.117	0.067	0.051	r
	UB	0.78	0.204	0.186	0.160	0.092	0.070	e

Pistachios

			Consumption					
			Spain	Germany	Ireland	France	UK	
ML	Occurrence		0.3	0.2	0.1	0.1	0.1	
4	LB	0.20	0.001	0.001	0.000	0.000	0.000	E
	UB	0.44	0.002	0.001	0.001	0.001	0.001	x
								p
8	LB	0.37	0.002	0.001	0.001	0.001	0.001	o
	UB	0.61	0.003	0.002	0.001	0.001	0.001	s
								u
10	LB	0.46	0.002	0.002	0.001	0.001	0.001	r
	UB	0.69	0.003	0.002	0.001	0.001	0.001	e

Sum for minimum of all countries/ regions (Ireland)

	ignoring other foods		adding other foods	
ML	exposure	increase	exposure	increase
4	0.064	-	0.611	-
8	0.095	49%	0.642	5.10%
10	0.118	84%	0.665	8.83%

Sum for maximum of all countries/ regions (Spain)

	ignoring other foods		adding other foods	
ML	exposure	increase	exposure	increase
4	0.144	-	2.042	-
8	0.185	28%	2.083	1.99%
10	0.212	47%	2.110	3.31%

Table 32: Changes in exposure calculations for **scenario 4 "high level exposure for pistachios"** for adult population by changing the MLs (LB=lower bound estimate, UB= upper bound estimate, consumption values are given as g/d, occurrence data and MLs as µg/kg, exposure values as ng/kg b.w. per day).

Almonds			Consumption					
			Spain	Germany	Ireland	France	UK	
ML	Occurrence		0.5	0.4	0.1	0.5	0.5	
4	LB	0.18	0.002	0.001	0.000	0.002	0.002	E
	UB	0.40	0.003	0.003	0.001	0.003	0.003	x
								p
8	LB	0.24	0.002	0.002	0 000	0.002	0.002	o
	UB	0.46	0.004	0.003	0.001	0.004	0.004	s
								u
10	LB	0.29	0.002	0.002	0.000	0.002	0.002	r
	UB	0.50	0.004	0.003	0.001	0.004	0.004	e

Hazelnuts			Consumption					
			Spain	Germany	Ireland	France	UK	
ML	Occurrence		0.1	1.4	0.2	0.4	0.2	
4	LB	0.31	0.001	0.007	0.001	0.002	0.001	E
	UB	0.53	0.001	0 012	0.002	0.004	0.002	x
								p
8	LB	0.46	0.001	0.011	0.002	0.003	0.002	o
	UB	0.68	0.001	0.016	0.002	0.005	0.002	s
								u
10	LB	0.57	0.001	0.013	0.002	0 004	0.002	r
	UB	0.78	0 001	0.018	0.003	0.005	0 003	e

Pistachios			Consumption					
			Spain	Germany	Ireland	France	UK	
ML	Occurrence		47.5	7.5	5.7	7.2	25.9	
4	LB	0.20	0.158	0.025	0.019	0.024	0.086	E
	UB	0.44	0.348	0.055	0.042	0.053	0.190	x
								p
8	LB	0.37	0.293	0.046	0.035	0.044	0.160	o
	UB	0.61	0.483	0.076	0.058	0.073	0 263	s
								u
10	LB	0.46	0.364	0.058	0.044	0.055	0.199	r
	UB	0.69	0.546	0.086	0.066	0.083	0.298	e

Sum for minimum of all countries/ regions (Ireland)

	ignoringother foods		adding other foods	
ML	exposure	increase	exposure	increase
4	0.020	-	0.567	-
8	0.037	82%	0.584	2.96%
10	0.046	127%	0.593	4.54%

Sum for maximum of all countries (Spain, upper bound)

	ignoring other foods		adding other foods	
ML	exposure	increase	exposure	increase
4	0.353	-	2.251	-
8	0.488	38%	2.386	6.01%
10	0.552	56%	2.450	8.85%

Table 33: Overview of maximal and minimal lower and upper bound exposure estimates in ng/kg b.w. per day.

Limit of truncation of occurrence data	Values below LOD	Scenario 1	Scenario 2	Scenario 3	Scenario 4
Minimal Case		Ireland	France	UK	Ireland
4	lower bound	0.548	0.560	0.576	0.567
	upper bound	1.080	1.106	1.129	1.121
8	lower bound	0.549	0.565	0.590	0.584
	upper bound	1.081	1.111	1.143	1.138
10	lower bound	0.550	0.569	0.601	0.593
	upper bound	1.082	1.114	1.153	1.146
Maximal Case		Spain	Spain	Spain	Spain
4	lower bound	0.822	0.887	0.903	0.980
	upper bound	1.904	2.047	2.042	2.251
8	lower bound	0.824	0.910	0.944	1.115
	upper bound	1.906	2.070	2.083	2.386
10	lower bound	0.825	0.928	0.973	1.187
	upper bound	1.907	2.085	2.110	2.450

4. Hazard characterisation

4.1 Summary of key data

4.1.1 Kinetics, distribution, metabolism and elimination

Absorption of aflatoxins in the rat small intestine is a rapid process that follows first-order kinetics, with an absorption rate constant [k(a)] of 5.84 ± 0.05 (AFB1), 4.06 ± 0.09 (AFB2), 2.09 ± 0.03 (AFG1) and 1.58 ± 0.04 (AFG2) h-1, respectively (Ramos and Hernández, 1996). Absorbed AFB1 reaches the liver through the portal system and is metabolised by cytochrome P450 (CYP)-dependent mono-oxygenases in reactions involving the incorporation of an atom of molecular oxygen into the substrate (Guengerich *et al.*, 1998). The resulting increase in polarity facilitates further metabolic processing and excretion (Figure 18).

The major aflatoxins occurring in human foods are AFB1, AFB2, AFG1 and AFG2 with AFM1 appearing as a metabolite in milk. Of these aflatoxins, the biological activity is mainly determined by the presence of a double bond at the 8,9-position of the molecule, permitting bioactivation to a reactive 8,9-epoxide. Only AFB1, AFG1 and AFM1 are therefore capable of being bioactivated by CYPs. Additional metabolites are formed from AFB1 following oxidation, including AFQ1 and AFM1 (see Figure 18) and the demethylated metabolite, AFP1. These metabolites and other naturally occurring aflatoxins (G1, B2, G2), being poorer substrates for epoxidation, are consequently less mutagenic, carcinogenic and toxic than AFB1. AFB1 metabolites can be useful biomarkers of human exposure to aflatoxins and AFM1, AFQ1 and AFP1 be excreted in

urine and bile and have all been detected in human urine samples (Groopman *et al.*, 1985).

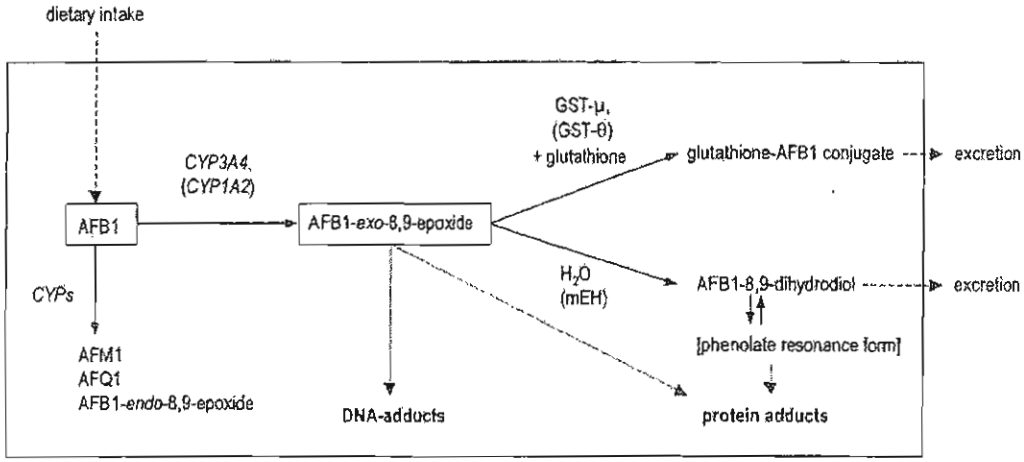

Figure 18: Schematic representation of AFB1 metabolism highlighting the formation of its critical product AFB1-*exo*-8,9-epoxide, its DNA- and protein adducts and major urinary metabolites (Tiemersma *et al.*, 2001).

The liver is the major site of aflatoxin metabolism and the CYPs that are implicated are CYP3A4, 3A5 and 1A2 (Wild and Turner 2002). There is a continuing discussion as to the most important CYPs with regard to aflatoxin metabolism in humans. CYP3A4 has been shown to catalyse the formation of the AFB1 *exo* 8,9-epoxide, which is able to bind to DNA, and AFQ1 whilst CYP1A2 can lead to the formation of some *exo*-epoxide as well as a high proportion of *endo*-epoxide, which does not bind to DNA and AFM1.

CYP1A2 has been reported to be more efficient in producing 8,9-epoxide at the low AFB1 concentrations that may be found following dietary exposures. The overall contribution of these enzymes to AFB1 metabolism *in vivo* will depend on affinity but also on expression levels in human liver, where CYP3A4 is predominant. CYP3A5 also metabolizes AFB1, mainly to the *exo*-8,9 epoxide, but it is much less efficient at forming the detoxification product, AFQ1 (Wang *et al.*, 1998). Recent more comprehensive comparisons between CYP expression and aflatoxin metabolism to the 8,9-epoxide in human liver samples have reported the primary importance of CYP3A4, and of CYP3A5 in livers with low CYP3A4 expression (Kamden *et al.*, 2006).

AFB1-8,9-dihydrodiol, resulting from hydrolysis of the 8,9-epoxide, is unstable and undergoes base-catalyzed rearrangement to a dialdehyde reacting with proteins, such as albumin, but not with DNA (Guengerich, 2005). The catalysis of AFB-8,9-epoxide hydrolysis by epoxide hydrolase has been proposed in the literature (Ch'ih *et al.*, 1983; McGlynn *et al.*, 1995; Kelley *et al.*, 2002). However, the rapid rate of non-enzymatic hydrolysis is difficult to compete with and therefore the contribution *in vivo* of this pathway remains unclear.

There are some data to suggest that HBV infection of the liver alters the expression of the genes coding for the enzymes which metabolise aflatoxins. For example, studies in HBV transgenic mouse lineages have revealed an induction of specific CYPs in association with liver injury resulting from expression of the HBV transgenes (Chemin *et al.*, 1996; 1999; Kirby *et al.*, 1994a). Induction of CYP enzymes has also been observed in mice and hamsters where liver injury was induced by infection with bacteria and parasites (Kirby *et al,* 1994b; Chomarat *et al,* 1997) suggesting a role for liver injury *per se* rather than a specific effect of HBV. Modulations of gene expression by liver injury may not be limited to CYP enzymes. A study of human liver specimens showed that GST activity is significantly decreased in the presence of HBV DNA (Zhou *et al,* 1997) and this is supported by data in human liver cells *in vitro* transfected with HBV (Jaitovitch-Groisman *et al.*, 2000), suggesting that viral infection may compromise the ability of hepatocytes to detoxify aflatoxins. One study assessed the impact of HBV infection on CYP activities in people exposed to aflatoxins in The Gambia (Wild *et al.*, 2000). Cortisol metabolism was used as a marker of CYP3A4 activity but no association was observed with HBV infection status. However, in West Africa higher levels of albumin adduct of aflatoxins have been observed in young children who were hepatitis B virus antigen positive ($HBsAg^+$) compared to those who were not (Wild *et al.*, 1993; Turner *et al.*, 2000). Similar observations have been reported in a study of 200 adolescents from Taiwan (Chen *et al.*, 2001) but not in Chinese adults (Wang *et al.*, 1996). Thus overall, effects of HBV infection on aflatoxin metabolism are likely to be complex, but there is potential for an altered balance of activation and detoxification during an infection. This may provide one mechanistic basis for the higher risk of liver cancer among HBV infected individuals exposed to aflatoxins.

4.1.2 DNA and protein adducts

The formation of macromolecular adducts by AFB1 depends on the balance between the rate of production of the AFB1-*exo*-8,9-epoxide compared to other oxidise metabolites and the rate of detoxication of the 8,9-epoxide via multiple biochemical pathways, including conjugation to glutathione and hydrolysis to the 8,9-dihydrodiol as described (section 4.1.1) (Essigmann *et al.*, 1982). The primary DNA adduct formed from the 8,9-epoxide is 8,9-dihydro-8-(N7-guanyl)-9-hydroxy-AFB1 (AFB1-N7-Gua) (Croy *et al.*, 1978, Croy and Wogan, 1981, see figure 19). AFB1-N7-Gua adduct can be converted into two secondary lesions, an apurinic (AP) site or the imidazole-ring opened AFB1-formamidopyrimidine (AFB1-FAPY) adduct. AFB1-FAPY adducts are detected at near maximal levels in rat DNA days to weeks after AFB1 exposure, underscoring its high persistence in vivo (Croy and Wogan, 1981; Smela *et al.*, 2002). AFG1-8,9-epoxide is formed but has a reduced ability to intercalate into the DNA helix because modification of the ring structure decreases planarity and therefore less adducts are formed for a given dose (Raney *et al.*, 1990).

In contrast to the persistence of the AFB1-FAPY, the half-life of the AFB1-N7-Gua, is of the order of a few hours. Metabolites of the adducts after repair are found in urine and is detectable in the urine of individuals exposed to aflatoxins in the diet in Africa and China (Groopman *et al.*, 1992; Groopman *et al.*, 1993).

As mentioned above, aflatoxins can also bind to proteins, such as albumin, via the formation of the AFB1-8,9-dihydrodiol (Wild et al, 1986; Sabbioni *et al.*, 1990). AFB1 and AFG1 dialdehydes form Schiff bases with primary amine groups e.g. lysine, to form aflatoxin-albumin (AF-alb) adducts (Sabbioni *et al.*, 1987; Sabbioni and Wild 1991). However, the formation of such protein adducts may be influenced by a further metabolic step involving aflatoxin aldehyde reductase (AFAR). This enzyme catalyzes the NADPH-dependent reduction of the dialdehydic phenolate form of AFB1-8,9-dihydrodiol to a dialcohol and would thus decrease the amount of AF-alb formed; this enzyme has been characterised in both rats and humans (Kelly *et al.*, 2004).

AF-alb accounts for around 2% of a single AFB1 dose in rat (3-1.200 µg AFB1/kg b.w.) and human studies (Wild *et al.*, 1986; Sabbioni *et al.*, 1990; Wild *et al.*, 1990). However in humans the adduct is estimated to accumulate up to 30-fold from chronic exposure because of the longer half-life of albumin compared to rats (~20 days compared to ~3 days). The only AFB1 adduct structurally identified to date in enzymatically digested plasma albumin is AFB1-lysine (Sabbioni *et al.*, 1987; Sabbioni 1990). The AF-alb adduct in serum and plasma together with urinary aflatoxin metabolites and adducts have been valuable biomarkers of aflatoxin exposure in epidemiological studies (Kensler *et al.*, 2003). There is a high correlation between the presences of aflatoxin-DNA adducts in the liver, their urinary excretion and the formation of the serum albumin adduct. Owing to their different half-lives, urinary and serum aflatoxin adduct levels reflect recent (1-2 days) and chronic (2-3 months) exposure, respectively.

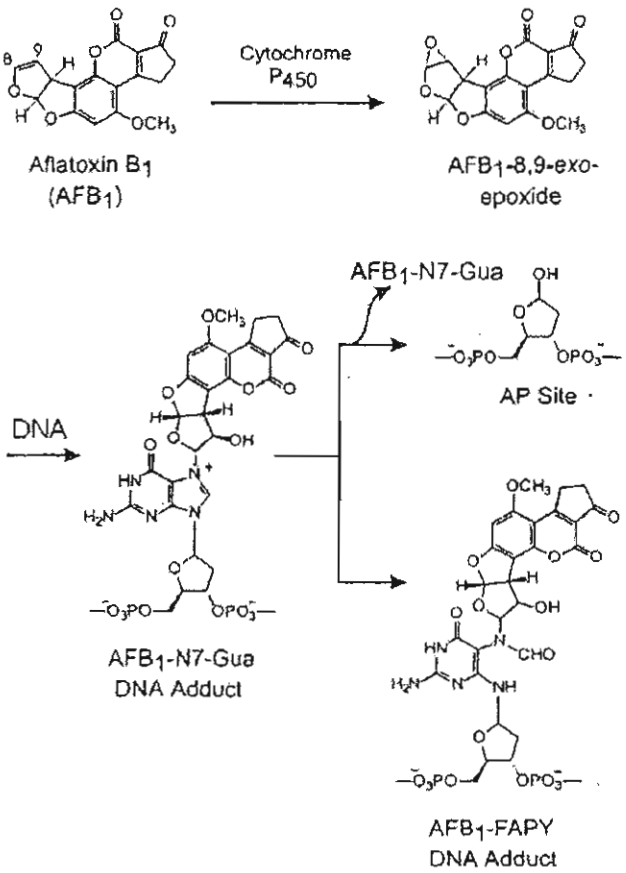

Figure 19: DNA lesions induced by AFB1 (from Smela *et al.*, 2001).

The availability of accelerator mass spectrometry for the sensitive detection of [^{14}C]-labelled carcinogen in human tissues has permitted studies of humans to known doses of aflatoxins (Cupid *et al.*, 2004). Human volunteers (n=7) were given a dose of about 15 ng/kg of [^{14}C] AFB1 about 3 to 7 hours before undergoing colon surgery. Blood and tissue samples were collected a few hours later to permit levels of aflatoxin binding to peripheral blood albumin and colon tissue DNA to be determined. Levels of AFB1-albumin adducts were similar to those in Fischer rats exposed to similar doses of AFB1. These direct observations may assist in relating biomarker data to aflatoxin exposure, but the number of subjects is small and all were aged cancer patients undergoing surgery. It is unclear therefore how reliable these data are for extrapolation to a general population.

4.1.3 DNA repair and mutagenicity

The repair mechanisms for AFB1-induced DNA damage are not well understood. Nucleotide excision repair (NER) plays a role in Escherichia coli and human cells (Sarasin *et al.*, 1977; Oleykowski *et al.*, 1993). However, human cells defective in NER

still repair a considerable amount of AFB1-DNA lesions (Waters *et al.*, 1992). Base excision repair as well as single- and double strand break repair, and recombinational and post-replication repair have been suggested to contribute also to the removal of AFB1-DNA lesions (reviewed in Bedard and Massey, 2006). These studies indicate that differences in DNA repair and modulation of repair by AFB1 are important contributing factors to both the tissue- and species-specific susceptibility to AFB1-induced carcinogenesis in murine models. Polymorphisms in DNA repair genes seem to play a role in hepatocellular carcinoma (HCC) risk of AFB1 exposed populations (Long *et al.*, 2006).

AFB1 is mutagenic in bacterial systems and in eukaryotes, usually requiring an exogenous bioactivation system (IARC, 1993). Many studies have defined the mutational spectrum produced after exposure of cells to AFB1 and the G>T transversion mutation is predominantly observed (reviewed in Sabbioni, 1990). The mutational properties of the AFB1-N7-Gua adduct have been studied in *E. coli* (Oleykowski *et al.*, 1993) where it primarily causes G>T mutations although at a very low frequency (4%). Recent studies indicate that the most likely candidate for mutagenicity by AFB1 is the AFB1-FAPY adduct (Sarasin *et al.*, 1977). This adduct causes a G>T mutation frequency in *E. coli* at higher frequency than the AFB1-N7-Gua adduct and it is a strong block to replication. Most HCC samples from people living in areas where HBV is prevalent have one mutational hotspot at codon 249 of the p53 gene that is a G>T transversion (Waters et al, 1992; Bedard and Massey, 2006).

4.1.4 Species differences

Despite several differences among species, short- and long-term experiments tend to support extrapolations across species with regard to the main mechanisms of toxicity and carcinogenicity and the use of biomarkers to predict both the risk of cancer and the outcome of chemo-prevention.

Notwithstanding the broad similarities in aflatoxin biotransformation across species, there are some key species differences regarding the affinity for and the catalytic activity of the main enzymes involved, particularly glutathione S-transferase (GSTs). GSTs are the main biosynthetic enzymes involved in AFB1-*exo*-8,9-epoxide conjugation, representing a major detoxication pathway. The order of GSH conjugation to AFB1 among species is mouse > rat > human with humans exhibiting comparatively low conjugation (Raney *et al.*, 1992; Kirby *et al.*, 1993). In animals experimentally treated with the same doses of AFB1 over a 14 day period, the level of AFB1-DNA and AFB1-albumin adducts was in the following order: rat > guinea pig > hamster > mouse (Wild *et al.*, 1996). Whilst mice produce relatively large amounts of exo-8,9-epoxide, they are highly resistant to AFB-hepatocarcinogenesis. This is because the mouse but not the rat expresses high constitutive levels of a hepatic alpha class GST, mGSTA3-3, with a high affinity for AFB1-8-9-epoxide (Buetler and Eaton 1992; Hayes *et al.*, 1992). There is however an

inducible alpha class GST (rGSTA5-5) in rats which can confer resistance to AFB1 through efficient conjugation of the 8,9-epoxide (Kensler *et al.*, 1986; Hayes *et al.*, 1991; Eaton *et al.*, 2001).

Human GST enzymes are therefore poor catalysts for the conjugation of AFB1 8,9-epoxide, except for GSTM1-1. Assuming that the catalytic activities of recombinant enzyme preparations reflect those in human tissue, the efficiency of the human GSTs vary over a range of 170-fold with regard to AFB1-8,9-epoxide conjugation (Table 34). The GSTM1-1 enzyme was the most efficient and was comparable to a rat ortholog, M3-3. This result is consistent with a study using human hepatocytes, in which AFB1-GSH conjugates were only detected in samples in which the GSTM1 enzyme was expressed (Langouët *et al.*, 1995).

The relevance of the estimated rates of conjugation is also related to the levels of individual GST expression in human liver; the interindividual variation is known to be considerable, probably reflecting both the inducibility of GSTs and the influence of genetic polymorphisms in the relevant genes. Moreover, GSH conjugation and subsequent formation of mercapturates in general is known to be a major metabolic pathway in rodents, whereas it is a minor one in humans. Despite this the detection of AFB1-mercapturic acid metabolites in human urine from aflatoxin-exposed individuals in China demonstrated that this conjugation pathway is active in people exposed to dietary aflatoxins (Wang *et al.*, 1999).

Table 34: Rates of GSH transferase-catalyzed conjugation of GSH with AFB *exo*-8,9-epoxide. Adapted from Johnson *et al.*, 1997.

GSH transferase	k_2 (s^{-1})	K (μM)	k_2/K, $M^{-1} s^{-1}$
Rat			
A10-10	3.2	1	63=10
M3-3	0.08	30	33=10
Human			
M1-1	0.055	30	$1.7=10^3$
T1-1	0.015	70	22=10
P1-1	0.002	20	210
A1-1	0.009	100	19=10
A2-2	0.001	100	10

Kinetic rate constants were estimated from iterative simulations of experimental determinations at various substrate and catalyst concentrations. The estimates are limited to F2 significant digits here.

4.1.5 Human variability and factors affecting biotransformation

As discussed above, the formation of macromolecular aflatoxin adducts depends on the balance between the rate of production of AFB1-*exo*-8,9-epoxide and its detoxication along three main pathways: (i) spontaneous or epoxide hydrolase-mediated hydrolysis; (ii) GSH conjugation; (iii) further oxidation by CYP450. Such pathways are therefore a potential source of inter-individual variation in susceptibility to aflatoxins.

With regard to the CYPs, CYP 3A4 and 3A5 are polymorphic and allele frequencies differ by ethnic group. For example, there is a relatively high frequency of some allelic variants of CYP3A4 in Afro-Americans compared to other ethnic groups (Table 35). Human hepatic CYP3A5 expression is polymorphic with a proportion of individuals showing no expression; in particular 40% of African-Americans do not express this enzyme. Recently, polymorphisms have been identified in the promoter region of CYP3A5 leading to alternative splicing and as truncated protein (Kuehl *et al.*, 2001; Hustert *et al.*, 2001). Furthermore, a study undertaken in China showed that CYP1A2 genetic polymorphisms are associated with HCC susceptibility in smokers and HBsAg seronegative individuals in the Fusui endemic region (Chen *et al.*, 2006). The prevalence of such variants in a given population and the functional significance in terms of aflatoxin metabolism would need to be understood in order to assess the potential impact on aflatoxin-associated disease risk. Where functional polymorphisms in key enzymes differ in prevalence by ethnic group, these could conceivably affect the impact of a given level of aflatoxin exposure at the population level.

Table 35: Allele frequency distribution of polymorphic genetic variants of CYP 3A4*1B by ethnic background. Adapted from Jernstrom *et al.*, 2001.

	White (*n* = 329)		Black (*n* = 78)		Asian (*n* = 71)		Indian-Pakistani (*n* = 25)	
	No. (%)		No. (%)		No. (%)		No. (%)	
CYP3A4								
No variant	305	(94.1%)	9	(11.5%)	69	(97.1%)	24	(100%)
Heterozygous variant	18	(5.6%)	38	(48.7%)	2	(2.8%)	—	
Homozygous variant	1	(0.3%)	31	(39.7%)	—		—	

Note. Missing values: Three *IGF1* alleles, four *AIB1* alleles, and five *CYP3A4* genotypes were missing among white women. *CYP3A4* genotype was missing for one Indian-Pakistani woman.

However, the most important contribution to inter-individual variability in CYPs is probably the phenotypic variation resulting from their known inducibility.

Moreover, compounds which induce these enzymes may also cause a competitive inhibition of metabolism of aflatoxins.

There have been a number of studies which have considered polymorphisms in aflatoxin metabolising or DNA repair enzymes in relation either to the formation of DNA or protein adducts or in relation to risk of HCC. Wild *et al.*, (1993) measured serum AF-alb in Gambian children in relation to GSTM1 genotype and in Gambian adults in relation to GSTM1, GSTT1, GSTP1 and epoxide hydrolase polymorphisms (Wild *et al.*, 2000) and found no major differences in adduct levels by genotype. Kensler *et al.*, (1998) found no association between AF-alb and GSTM1 genotype in adults from Qidong County, People's Republic of China. More recently, however, CYP3A5 genotypes associated with high expression were shown to have higher AF- alb levels in Gambian adults (Wojnowski *et al.*, 2004).

The possibility that polymorphisms in DNA repair enzymes could affect the levels of AFB1-N7-Gua adducts has been less extensively studied. Lunn *et al.* (1999) examined the levels of AFB1-DNA adducts in placental DNA from Taiwanese mothers in relation to polymorphisms in the DNA repair enzyme, XRCC1. The presence of at least one allele of polymorphism 399Gln was associated with a 2-3-fold higher risk of detectable AFB1-DNA adducts.

In terms of studies of polymorphisms and HCC, a number of studies have been published. A recent case–control study including 257 HCC cases and 649 hospital-based age, sex, ethnicity, and hepatitis B virus infection-matched controls was carried out to examine the role of genetic polymorphisms of four genes (GSTM1, GSTT1, HYL1*2, and XRCC1) in the Guangxi population (Long *et al.*, 2006). A significantly increased risk was associated with a combination of "at-risk" genotypes [GSTM1-null, HYL1*2-YH/HH, and XRCC1-AG/GG], particularly in those estimated to be exposed to high levels of aflatoxins. In a parallel study in West Africa, Kirk *et al.*, (2005a) also reported a higher risk of HCC and some evidence of a greater effect in those exposed to higher amounts of aflatoxins. Despite the rapid rate of non-enzymatic hydrolysis, which seems to occur without the intervention of epoxide hydrolase, in one case–control study, mutant alleles of epoxide hydrolase were significantly over-represented in persons with HCC (McGlynn *et al.*, 1995).

As a whole, these data suggest that caution must be exercised in risk assessment, as the risk of cancer depends on a number of environmental factors and host characteristics, either genetically determined or acquired. Within such complex traits it may be even difficult to identify a "reference" population and hence any control estimate. It is also clear that large relative risk obtained after stratification by multiple factors simultaneously considered are usually unstable from a statistical viewpoint, due to small sample size. Large relative risks are compensated by large confidence intervals and associated uncertainty of estimates.

4.1.6 Toxicity and carcinogenicity

Aflatoxins are well recognized as a cause of liver cancer but they have additional important toxic effects with a range of consequences: 1) large doses lead to acute toxicity and death; 2) chronic sublethal doses have nutritional and immunologic consequences.

4.1.6.1 Acute toxicity

AFB1 causes acute hepatotoxicity in humans and experimental animals. Animal studies have found two orders of magnitude difference in the median lethal dose for AFB1. The most susceptible species are rabbits and ducks while chickens and rats have greater tolerance. Wong and Hsieh (1980) reported the oral LD50 in rat models as ranging from 5.5 to 17.9 mg/kg b.w. AFB1.

There have been a few reports of human poisoning with aflatoxins (Hall and Wild 1994), most recently in two consecutive years in Kenya (Lewis *et al.*, 2005) In April 2004, one of the largest aflatoxicosis outbreaks occurred, resulting in 317 cases and 125 deaths. Fifty-five percent of maize products from markets and maize vendors in the affected areas had aflatoxin levels greater than the Kenyan regulatory limit of 20 µg/kg; 35% had levels >100 µg/kg and 7% had levels >1,000 µg/kg. Makueni, the district with the most aflatoxicosis case-patients, had significantly higher market maize aflatoxins than did Thika, the study district with fewest case-patients (geometric mean aflatoxin=52.91 µg/kg vs. 7.52 µg/kg, p=0.0004). A case-control study conducted on patients with acute aflatoxicosis showed AFB1-lysine adducts the highest ever reported concentrations of AFB1-lysine adducts at or above 0.25 ng of AFB1-lysine per mg of albumin were a risk factor for developing aflatoxicosis) (Azziz-Baumgartner *et al.*, 2005).

4.1.6.2 Immune suppression, nutrition and growth effects

In animal experiments AFB1 has been shown to induce thymic aplasia, reduce T-lymphocyte function and number, suppress phagocytic activity and reduce complement activity (reviewed in Williams *et al.*, 2004). Many studies conducted in poultry, pigs and rats showed that exposure to aflatoxins results in suppression of the cell-mediated immune response. Several reports suggest that aflatoxins impair the function of macrophages in animal species. The species differences noted for the acute toxicity and carcinogenicity also apply to the immune response. Table 36 summarizes data relative to the effects of AFB1 on the immune system in mice and rats. The NOAELs are mostly in the region of 30 µg/kg b.w. Studies on Gambian children (Turner *et al.*, 2003) and Ghanaians (Jiang *et al.*, 2005) indicate that dietary exposure to AFB1 could result in impairment of cellular immunity that could decrease host resistance to infections.

Chronic aflatoxin exposure has major effects on nutritional status in animals but thresholds for these effects are not defined for any species. Recent studies described a dose-response relationship between aflatoxin exposure and the degree of stunting and underweight in children < 5 yrs old in Benin and Togo exposed to aflatoxin (aflatoxin-

albumin adducts range 5-1064 pg/mg albumin in 99% of the children) Gong *et al.*, 2002; 2004).

Table 36: Effects of AFB1 on immune response in murine systems.

Strain	Dose of AFB1	Duration of dosing	Effect on immune response	NOAEL µg/kg b.w. per day	LOAEL µg/kg b.w. per day	Comment	Ref.
Male CD-1 mice	30, 145, 700 µg/kg b.w	Alternate days for 2 weeks	Decrease in T-lymphocyte function and number	30	145		Reddy *et al.*, 1987
			Impaired DTH	30	145		
Male BALB/c mice	30, 145, 700 µg/kg b.w	Alternate days for 4 weeks	Decrease in lymphocyte number	30	145		Reddy *et al.*, 1989
			Inhibition of NK cell response	-	30		
Male C57B1/6 mice	30, 150, 750 µg/kg b.w	Daily for 4 weeks	Decrease in splenic CD4 cell number and IL-2 production	30	150		Hatori *et al.*, 1991
Male weanling rats	60, 300, 600 µg/kg b.w.	Alternate days for 4 weeks	Impaired DTH	60	300		Raisuddin *et al.*, 1993
			Proliferative response of B-cells	60	300		
			Proliferative response of T-cells	-	60		
Male Wistar rats	40 µg/kg b.w.	90 days	Higher mitogenic response and altered production of IL-2 and IL-4 by SMC			Total intake/kg b.w. at day 90: 354 µg	Theumer *et al.*, 2003
Male Fischer 344/NHla rats	Aerosol inhalation 3.17 µg/l		Suppression of alveolar macrophage phagocytosis			Aerosol inhalation estimated dose: 16.8 µg/kg b.w.	Jakab *et al.*, 1994
Female Swiss mice	Intratracheal instillation 12.5 to 150 µg						

DTH= delayed type hypersensitivity
SMC= spleen mononuclear cells

4.1.6.3 Long term toxicity and carcinogenicity

Studies have consistently shown AFB1 to be both genotoxic and carcinogenic in experimental animals. Sufficient experimental evidence is also available for the carcinogenicity of naturally occurring mixtures of aflatoxins, and of AFG1 and AFM1, whereas there is only limited evidence for AFB2 and inadequate evidence for AFG2 (FAO/WHO, 1998; IARC, 1993 and 2002).

Aflatoxin B1

For the carcinogenic effect of AFB1 there are species and strain differences in sensitivity, which may arise from differences in the rates and extents of metabolic activation and detoxification (see section 4.1.3). In rodents, the principal tumours were in the liver, primarily HCC, but tumours were also found in aflatoxin-treated animals at other sites including the lung, kidney, and colon. The effective dose of AFB1 for induction of liver tumours varied over a wide range in different animal species when AFB1 was administered by continuous feeding, generally for the lifetime of the animal. Effective doses were 10-30 µg/kg in the diet in fish and birds. Rats responded according to strain at levels of 15-1000 µg/kg diet and in addition to tumours of the liver, tumours were also induced in the kidneys and the colon. The mean TD_{50} value based on various studies in male rats was reported to be 3.2 µg/kg b.w. per day (CPD, 2006). The TD_{50} was defined by Gold *et al.* (1984) and Peto *et al.* (1984) as follows: "For any particular sex, strain, species and set of experimental conditions, the TD_{50} is the dose rate (in mg/kg b.w. per day) that, if administered chronically for a standard period – the "standard lifespan" of the species – will halve the mortality-corrected estimate of the probability of remaining tumourless throughout that period". A particularly wide variation in sensitivity has been seen in mice with TD_{50} >70 µg/kg b.w. per day in C3H and C57BL mice and >5300 µg/kg b.w. per day in Swiss mice, and some strains of mice showing no response at doses up to 150000 µg/kg diet. Tree shrews responded to 2000 µg/kg diet with liver tumours; the TD_{50} was reported to be 26.9 µg/kg b.w. per day. In subhuman primate species, AFB1 potency in induction of liver tumours differed widely. Squirrel monkeys developed liver tumours when fed AFB1 at 2000 µg/kg diet for 13 months, and rhesus, African green and Cynomolgus monkeys developed a low (7-20%) incidence of liver tumours when fed average doses of 99-1225 mg/animal over 28-179 months. In these species tumours in extrahepatic tissues (including tumours of the pancreas, gall bladder, and the vascular system) were observed at much higher frequency than the liver tumours. The TD_{50} for liver tumours in rhesus monkeys was 156 µg/kg b.w. per day and for all tumours combined it was 8.2 µg/kg b.w. per day. In the Cynomolgus monkeys the TD_{50} for liver tumours was 848 µg/kg b.w. per day and for all tumours combined it was 20.1 µg/kg b.w. per day. (Wogan, 1992; FAO/WHO, 1998; CPD, 2006).

From the available data it can be concluded that some strains of rats are particularly sensitive to the liver carcinogenicity of AFB1. Studies in which AFB1 was given to rats using dietary administration are presented in Table 37. From this table and the TD_{50} values calculated for some studies, it appears that the Fisher rat is the most sensitive strain and that males are slightly more sensitive than females.

Aflatoxin B2

AFB2 has not been studied extensively. AFB2 binds to DNA of rats treated *in vivo*, after its metabolic conversion to AFB1. In rodent cells, AFB2 induced DNA damage, sister chromatid exchange and cell transformation, but not gene mutations. AFB2 produced gene mutations in bacteria (FAO/WHO 1998). IARC (1976) judged AFB2 to be about 100 times less potent than AFB1 but concluded in 1993 that there is limited evidence for carcinogenicity of AFB2 in experimental animals (IARC, 1993).

Table 37: Induction of liver tumours (mainly HCC) in rats after dietary administration of AFB1.

Sex/strain	Dose µg/kg b.w. per day	Duration of dosing	Tumour incidence	Comments	Reference
Male/Fisher rats	0	80 w	0/25		Wogan and Newberne, 1967
	0.75	68 w	12/12		
	15	35-52 w	6/20		
	50	35-41 w	18/22		
	50	2 w	1/16	After 82 w	
Female/Fisher rats	0	80 w	0/25		Wogan and Newberne, 1967
	0.75	80 w	13/13		
	15	60-70 w	11/11		
	50	64 w	4/4		
	50	2 w	1/13	After 82 w	
Female/Porton rats	0	104 w	0/34	TD_{50} = 12.5 µg/kg b.w./day	Butler and Barnes, 1968
	5	104 w	5/30		
	25	104 w	26/33		
Male/Porton rats	0	104 w	0/46	TD_{50} = 3.52 µg/kg b.w. /day	Butler and Barnes, 1968
	4	104 w	17/34		
	20	104 w	25/25		
Male/Wistar rats	0	147 d	0/24		Epstein *et al.*, 1969
	12.5	147 d	8/13	After 742 days	
	25	147 d	13/18	After 622 days	
	50	147 d	12/14	After 611 days	
Male/CDR rat	0	104 w	0/50	TD_{50} = 4.19 µg/kg b.w. /day	Newberne and Rogers, 1973
	4	104 w	24/50		
Female/Fisher rats	0	104 w	0/15	TD_{50} = 9.93 µg/kg b.w./day	Nixon *et al.*, 1974
	1	104 w	1/15		
Male/Fisher rats	0	104 w	0/16	TD_{50} = 1.13 µg/kg b.w. /day	Nixon *et al.*, 1974
	0.8	104 w	5/13		
Male/Fisher rats	0		0/18	TD_{50}= 0.932 µg/kg b.w. /day	Wogan *et al.*, 1974
	0.04	104 w	2/22		
	0.2	93 w	1/22		
	0.6	96 w	4/21		
	2.0	82 w	20/25		
	4.0	54 w	28/28		
Female/F344 rats	0	104 w	0/144	TD_{50} = 50.7 µg/kg b.w./day	Elashoff *et al.*, 1987
	0.25	104 w	0/24		
	0.75	104 w	0/24		
	2.25	104 w	1/24		
Male/F344 rats	0		1/144	TD_{50} = 49.9 µg/kg b.w./day	Elashoff *et al.*, 1987
	0.2		0/23		
	0.6		0/24		
	1.8		1/23		

Aflatoxin G1

AFG1 binds to DNA and produces chromosomal aberrations in bone-marrow cells of rodents treated *in vivo*. *In vitro* it induces mutations and chromosomal damage in bacteria and causes DMA damage and chromosomal abberrations in cultured human and animal cells (FAO/WHO 1998). AFG1 is almost as potent as AFB1 in producing liver tumours in trouts and rats. In trouts, Halver (1968) found 1.5 to 4 times fewer tumours in the AFG1-dosed than in the AFB1-dosed trouts after 16 months of dosing. In rats, equimolar doses of AFG1 caused about 5 times fewer liver tumours than AFB1 in both sexes (Butler *et al.*, 1969). However, AFG1 induced a higher incidence of kidney tumours than AFB1 and should therefore be considered to be equally potent to AFB1. IARC concluded in 1993 that there was sufficient evidence in experimental animals for the carcinogenicity of AFG1.

Aflatoxin G2

AFG2 has been the subject of very little research. From consideration of the chemical structures it may be reasonable to assume that AFG2 would be of similar potency to AFB2. IARC concluded in 1993 that there was inadequate evidence for the carcinogenicity of AFG2.

Aflatoxin M1

AFM1 is a metabolic hydroxylation product of AFB1. AFM1 is considered to be a genotoxic agent, based on its activity *in vitro* and its structural similarity with AFB1 (FAO/WHO 2001). In an experiment with the same dose of AFM1 as AFB1 to Fisher rats (total dose 1mg per rat), tumours developed considerably faster in the animals dosed with AFB1 than in those dosed with AFM1. After 2 years all AFB1 dosed animals had liver tumours, but only 1 of 29 AFM1-dosed animals (3%). However, 28% of AFM1-dosed animals showed preneoplastic liver lesions (Wogan and Paglialunga, 1974). In another study, groups of Fisher rats were maintained on diets containing AFM1 at 0, 0.5, 5, or 50 µg/kg and were killed between 18 and 22 months. In rats fed the diet containing AFM1 at 50 µg/kg, hepatocellular carcinomas were detected in two of 18 rats killed at 21 months, and neoplastic nodules were found in six of 37 rats killed between 19 and 21 months. No nodules or carcinomas were observed in the groups receiving the lower doses. Nineteen of 20 rats fed a diet containing AFB1 at 50 µg/kg had developed hepatocellular carcinomas by 17 months (Cullen *et al.*, 1987). From these results, FAO/WHO (2001) as a conservative estimate, considered the potency of AFM1 to be 10% that of AFB1. IARC concluded in 1993 that there was sufficient evidence in experimental animals for the carcinogenicity of AFM1 and inadequate evidence for the carcinogenicity of AFM1 in humans.

4.1.7 Human epidemiological data

4.1.7.1 Hepatocellular carcinoma incidence worldwide

HCC is the sixth most common cancer worldwide with 626,000 (5.7% of total) new cases in 2002 (Parkin *et al.*, 2005). Mortality is almost synonymous with incidence, given the poor survival rates; in 2002 there were 598,000 deaths in the world from HCC.

The majority of HCC cases (>80%) occur in developing countries with a higher incidence in males compared to females. In Europe the incidence rates for HCC are generally low, other than in southern Europe where they are intermediate compared to other areas of the world (Bosch and Ribes, 2000 ; Levi *et al.*, 2004 a, b; Parkin *et al.*, 2005, (Table 38). There is however, some evidence of an increase in incidence of HCC in parts of Europe (Levi *et al.*, 2004 a, b)

Table 38: Hepatocellular carcinoma rates in Europe.

Region	Age standardised rate (ASR) per 100,000 per year	
	Males	Females
Southern Europe	11.6	4.0
Western Europe	6.2	1.7
Eastern Europe	5.3	2.4
Northern Europe	3.4	1.7

4.1.7.2 Aetiology of hepatocellular carcinoma

The epidemiology of hepatocellular carcinoma in relation to exposure to chronic infection with hepatitis B virus (HBV) and aflatoxins has been reviewed in depth on a number of occasions. The International Agency for Research on Cancer (IARC) classified naturally occurring aflatoxins as human carcinogens in 1987 and again in 1993 (IARC 1987, 1993); IARC updated their evaluation in 2002 (IARC, 2002). The majority of the early epidemiological studies of aflatoxins and liver cancer comprised ecological or case: control studies that did not take account of HBV infection (IARC 1987, 1993). In addition, aflatoxin exposure is notoriously difficult to measure at the individual level. Aflatoxins are heterogeneously distributed in food commodities such as maize and groundnuts causing difficulties in obtaining representative samples for analysis. In addition, this heterogeneity means that questionnaires concerning consumption of foods commonly contaminated with aflatoxins are relatively uninformative. Nevertheless, reasonably consistent associations were found between estimates of dietary exposure to aflatoxins and HCC rates in a number of countries in sub-Saharan Africa and south-east Asia (IARC 1993).

In Europe, no epidemiological studies have directly investigated aflatoxin exposure as a risk factor for HCC. In terms of other HCC risk factors in Europe, 28% of liver cancer

cases have been attributed to chronic HBV infection and 21% to HCV infection. Other risk factors have included alcohol consumption, cigarette smoking and oral contraceptives (Bosch and Ribes, 2000). A more recent meta-analysis focused on risk factors for HCC in southern Europe, reporting that about 85% of all HCC cases were attributable to alcohol and infections with HBV or HCV leaving "little or no room to other already known risk factors, such as haemochromatosis and other genetic diseases, and to new, still unrecognized factors" (Donato *et al.*, 2006).

From the 1990s onwards epidemiological evidence for a role for aflatoxins in the aetiology of HCC has come from two main sources, both involving biomarkers. Namely, a number of longitudinal studies have incorporated aflatoxin biomarkers to measure aflatoxin exposure at the individual level whilst some molecular analyses have provided mechanistic evidence for a link between aflatoxins and HCC aetiology. A number of the relevant studies are summarised in Table 38.

Cohort studies

There were two longitudinal cohort studies of HCC using urinary (aflatoxin metabolites) and blood (aflatoxin-albumin) based biomarkers to assess individual aflatoxin exposure that showed significant interactions with chronic HBV infection (Ross *et al.*, 1992; Qian *et al.*, 1994; Wang *et al.*, 1996). Both studies, in Shanghai (Ross *et al.*, 1992; Qian *et al.*, 1994) and Taiwan (Wang *et al.*, 1996), reported increased risks of HCC in individuals positive for HBV infection and aflatoxin biomarkers alone, but a more than multiplicative interaction between the two risk factors (see Table 38). In a follow-up of the cohort reported by Wang *et al.*, (1996), Sun and co-workers (2001) compared aflatoxin-albumin adducts in hepatitis B virus antigen (HBsAg) carriers who had developed HCC compared to those that had not. There was a statistically significant relationship between detectable AFB1-albumin adducts and HCC risk (Table 39).

Yu *et al.*, (1997) analysed a number of urinary aflatoxin biomarkers in a nested case: control study of HCC in Taiwan. As all cases except one were HBsAg positive, they matched cases with HBsAg positive controls from the cohort and observed an OR of 6.0 for those with the highest urinary AFM1 levels; other urinary aflatoxin biomarkers were not associated with an increased OR. Other similar analyses of aflatoxin-related risk among HBsAg carriers have been reported by Sun *et al.*, (1999) and Chen *et al.*, (1996) (see Table 39).

A relatively consistent qualitative picture emerges from these studies, of a higher risk of HCC in individuals chronically infected with HBV and exposed to higher levels of aflatoxins, compared to individuals either not chronically infected with HBV and exposed to aflatoxins or infected with HBV but exposed to lower levels of aflatoxins. However, it is notable that none of the above studies provided direct quantitative relationships between dietary aflatoxin intakes, biomarkers and HCC risk. Aflatoxin biomarker

exposure data were typically reported categorically, as positive or negative or high and low.

In order to use more recent epidemiological studies that have employed biomarkers (see Table 39) for risk assessment there would need to be a well-defined quantitative relationship between the biomarker and the aflatoxin intake at the individual level. Although some studies have addressed this (Gan *et al.*, 1998; Wild *et al.*, 1992; Groopman *et al.*, 1992), these studies tend to be on a small scale in one specific population. In addition, in the published papers using these biomarkers to examine liver cancer risk in epidemiological studies, the biomarker data are often presented in categorical fashion (e.g. exposed/non-exposed; high exposure/low exposure) and hence the quantitative data on an individual basis are unavailable in the public domain.

Table 39: Studies of the interaction between aflatoxins and HBV in HCC.

Ref.	Population	Cohort	Cases	Controls	Biomarker	OR
Qian *et al.*, 1994	Shanghai, PRC	18,224 males	50	267	Urinary AF biomarker [d]	3.4 (1.1-10.0) AF alone 7.3 (2.2-24) HBsAg [h] alone 59.4 (16.6-212) AF and HBsAg
Wang *et al.*, 1996	Taiwan	12,040 males 13,758 females	56	220	Urinary AF metabolites [e]	1.7 (0.3-10.8) AF alone 22.8 (3.6-143.4) HBsAg alone 111.9 (13.8-905) AF and HBsAg
			29 HBsAg$^+$ [i]	21 HBsAg$^+$	Urinary AF metabolites [f]	5.5 (1.3-23.4)
Chen *et al.*, 1996	Taiwan	6,487 4,691 males 1,796 females	33 (20)	123 (86) [g]	AFB1-albumin adducts	5.5 (1.2-24.5) AF alone 129 (25-659) AF and HBsAg
Yu *et al.*, 1997	Taiwan	7,342 males 4,841 HBsAg carriers 2,501 non-carriers	43 HBsAg$^+$	86 HBsAg$^+$	Urinary AFM1	6.0 (1.2– 29.0) [a]
Sun *et al.*, 2001	Taiwan	12,024 males 13,594 females	79 HBsAg$^+$	149 HBsAg$^+$	Serum AFB1-albumin	2.0 (1.1-3.7) [b]
Sun *et al.*, 1999	Qidong Co., PRC	145 male HBsAg carriers	22 HBsAg$^+$	123 HBsAg$^+$	Urinary AFM1 [c]	3.3 (1.2-8.7)

a) Highest compared to lowest tertile of AFM1 level; adjusted for educational level, ethnicity, alcohol, cigarette smoking
b) Detectable versus non-detectable; adjusted for sex, age and residence
c) Eight monthly urine samples were collected over follow-up and urinary AFM1 analysis was conducted on a pooled sample; AFM1 positive compared to negative
d) Presence versus absence of any aflatoxin biomarker; adjusted for cigarette smoking
e) Low versus high urinary aflatoxin biomarker; adjusted for cigarette smoking and alcohol drinking
f) Low versus high urinary aflatoxin biomarker; adjusted for age, residence, cigarette smoking and alcohol drinking
g) Only the numbers of subjects in brackets had samples for analysis of aflatoxin biomarker;
h) Hepatitis B virus antigen
i) Hepatitis B virus antigen positive

Molecular analyses

The biological plausibility of the association between aflatoxin exposure and HCC risk is supported by the association between exposure and a specific point mutation in the third nucleotide of codon 249 (AGG to AGT) of the TP53 tumour suppressor gene (codon 249^{ser} mutation) (Wild and Turner, 2002). This particular type of transversion mutation at guanine residues is consistent with that induced by aflatoxins in a variety of experimental models. Overall, published studies show a positive correlation between population estimates of aflatoxin exposure and the proportion of HCC with a 249^{ser} mutation (Wild and Turner, 2002). In regions of China where aflatoxin exposure is reported as high, the 249^{ser} mutation was observed in more than 50% of HCC compared to less than 10% in low exposure regions. In geographic regions of expected low aflatoxin exposure (including Europe and North America) the prevalence of 249^{ser} mutations is extremely low (<1%).

Chronic HBV infection is generally considered insufficient to cause the 249^{ser} mutation, because of the extremely low prevalence of mutations in HBV infected people with HCC from North America, Europe and Japan (Lasky and Magder, 1997). The absence of the 249^{ser} mutation in HCC from Europe is therefore consistent with a limited role for aflatoxins in the disease in this region. However, the number of HCC examined for the mutation is relatively few. For example in the summary by Lasky and Madger (1997) only 71 HCC from Europe are listed of which one had a 249^{ser} mutation. In addition, aflatoxins could contribute to HCC by mechanisms other than TP53 mutation.

The high prevalence of HBV infection in aflatoxin endemic areas has made it more difficult to define whether both risk factors are required for the 249^{ser} mutation to occur. In the meta-analysis by Lasky and Madger (1997) data were available on 449 patients, 201 positive for HBV markers and 248 negative. The association between level of aflatoxin exposure and 249^{ser} mutation was still observed when restricting the analysis to HBV positive patients in high and low aflatoxin exposure groups. However, the number of HBV negative patients with high aflatoxin exposure was too small to make a similar comparison in HBV negative cases.

Case: control studies

Case: control studies of aflatoxins and HCC have been conducted in sub-Saharan Africa. It is noteworthy that no prospective cohort studies of HBV, aflatoxins and HCC have been reported in this region of the world.

Omer *et al.*, (2004) conducted a case: control study of HCC in Sudan and assessed peanut consumption, as a surrogate for aflatoxin exposure in this population, and HBV infection. There was a significant association with peanut butter consumption and HBV infection, and a more than additive interaction between the two was reported. Whilst this study had

the positive feature of confirming HCC by histology in 95% of cases, the use of peanut consumption as a surrogate for aflatoxin exposure is a significant limitation. In line with the studies in China and Taiwan, no estimates of dietary aflatoxin exposure in relation to HCC risk were made.

Kirk *et al.*, (2004; 2005b) reported a case: control study of HCC in The Gambia. Both HBV and HCV infection were significantly associated with HCC risk, albeit HCV infection rates were low. In a follow-up analysis the authors examined the 249^{ser} mutation in the plasma of HCC cases, cirrhosis patients and controls (Kirk *et al.*, (2005b). They found the mutation detectable in 39.8, 15.3 and 3.5% of the three groups respectively with an OR of 20.3 (8.19-50.0) for individuals positive for the mutation in plasma. Furthermore, the presence of both the 249^{ser} mutation and HBV infection was associated with an OR = 399 (48.6-3270). These data are consistent with a multiplicative effect on HCC risk of the mutational effect of aflatoxin on TP53 and chronic infection with HBV. It is unclear to date whether the 249^{ser} mutation is simply a marker of HCC or a measure of aflatoxin exposure. However, this study again provides no information on aflatoxin exposure level in relation to HCC risk.

4.1.7.3 Hepatitis B virus prevalence

Given the above discussion it is clearly important to consider the prevalence of HBV in a population in relation to the associated population risks from aflatoxin exposure. The WHO has presented data on chronic HBV infection world-wide in relation to three areas where the prevalence of infection is: high (>8%), intermediate (2-8%), or low (<2%) (Mahoney, and Kane, 1999; Viral Hepatitis Prevention Board 1998). Areas with high endemicity include south-east Asia and the Pacific Basin (excluding Japan, Australia, and New Zealand), sub-Saharan Africa, the Amazon Basin, parts of the Middle East, the central Asian Republics, and some countries in Eastern Europe.

In general in eastern and southern (Mediterranean region) Europe HBV carriage rates are between 2-7% (see Table 40) whilst in the rest of Europe they are below 1% (see Table 40) and less than 20% of the population is ever exposed to HBV infection. However, as noted the prevalence of chronic infection is considered endemic in some regions, especially the Central Asian Republics and some Eastern European countries including Bulgaria, Romania, Albania and Moldova, where carriage rates can be >8% (Meheus, 1998; Maddrey 2000).

Table 40: Prevalence of hepatitis B in various areas. Adapted from World Health Organisation 2001.

Area	% of population positive for infection			
	HBsAg	anti-HBs	Neonatal	Childhood
Northern, Western, and Central Europe, North America, Australia	0.2-0.5	4-6	Rare	Infrequent
Eastern Europe, the Mediterranean, Russia and the Russian Federation, Southwest Asia, Central and South America	2-7	20-55	Frequent	Frequent
Parts of China, Southeast Asia, tropical Africa	8-20	70-95	Very frequent	Very frequent

From: Zuckerman AJ. Hepatitis Viruses. In: Baron S, eds. Medical Microbiology, 4th ed. Galveston, TX, The University of Texas Medical Branch at Galveston, 1996:849-863.

There are also specific high risk groups within European populations, including drug users and immigrants from areas of the world with high prevalence rates. As an example, in Germany, an area of low HBV infection, there are 7.3 million foreign citizens and 3.2 million immigrants from the former USSR and Eastern Europe, many of which are from regions with high HBV infection rates (Marschall *et al.*, 2005).

Overall, it has been difficult to identify a systematic review of HBV prevalence rates in Europe from the scientific literature. There is a need for a systematic review of HBV and also HCV infection chronic carrier rates in EU countries, given that immigration rates in some new member countries are leading to dynamic changes in prevalence rates for these infections. Studies of liver cancer incidence in populations where HBV vaccination has been implemented for a number of years may inform quantitative risk assessment in the future.

4.1.8 Dose-response modelling

The Panel considered the liver carcinogenicity of aflatoxins to be the pivotal effect for the risk assessment. Studies have consistently shown AFB1 to be both genotoxic and carcinogenic in experimental animals. Sufficient experimental evidence is also available for the carcinogenicity of naturally occurring mixtures of aflatoxins, and of AFG1 and AFM1, whereas there is only limited evidence for AFB2 and inadequate evidence for AFG2 (FAO/WHO, 1998; IARC, 1993 and 2002).

The potential carcinogenicity in humans of the aflatoxins (either total or AFB1) has been examined in a large number of epidemiology studies, generally carried out in Africa and Asia, where substantial quantities of aflatoxins occur in basic foodstuffs. Exposure to aflatoxins appears to present an additional risk, which is enhanced by simultaneous

exposure to hepatitis B virus, and possibly hepatitis C virus (FAO/WHO, 1998; IARC 1993 and 2002).

Because aflatoxins are both genotoxic and carcinogenic the Panel could not establish a no observed adverse effect level (NOAEL) as a point of departure for the risk assessment. Therefore, the Panel considered dose-response modelling of experimental data from animal experiments and data from epidemiological studies. The Panel noted that the available database for dose-response modelling would only be sufficient for AFB1. Therefore, and taking into account that AFG1 and AFB2 were also shown to be carcinogenic in rodents, albeit at lower potency than AFB1, in the risk characterisation the Panel as a conservative approach assumed that the carcinogenic potency of "total aflatoxins" would be similar to that of AFB1.

Aflatoxin B1

Benchmark dose (BMD) modelling of animal data

The BMD approach was originally put forward by Crump (1984) as an alternative to the NOAEL and LOAEL for non-cancer health effects because it provides a more quantitative alternative to the first step in the dose-response assessment than the NOAEL/LOAEL. The BMD is based on a mathematical model being fitted to the experimental data within the observable range and estimates the dose that causes a low but measurable response (the benchmark response BMR) typically chosen at a 5 or 10% incidence above the control. The BMD lower limit (BMDL) refers to the corresponding lower limits of a one-sided 95% confidence interval on the BMD. Using the lower bound takes into account the uncertainty inherent in a given study, and assures (with 95% confidence) that the chosen BMR is not exceeded.

For the evaluation of human and experimental animal data the EFSA Scientific Committee has proposed to use the BMD methodology to derive a reference point on the dose-response curve. The Scientific Committee was of the opinion that the use of the BMDL, calculated for a BMR of 10% (BMDL10), is an appropriate reference point for compounds that are both genotoxic and carcinogenic. Such a value is the lowest statistically significant increased incidence that can be measured in most studies, and would normally require little or no extrapolation outside the observed experimental data.

Although a number of carcinogenicity studies of AFB1 have been performed in animals, most of them are not suitable for dose-response modelling for the risk assessment of AFB1 in food either because they did not use dietary administration, used only a single dose, or produced a 100% response in all dosed groups. In addition, in many studies aflatoxin administration was combined with some other treatment. The animal species most sensitive to the liver carcinogenicity of AFB1 appears to be the rat. The Panel considered the study in male Fisher rats performed by Wogan *et al.* (1974) as the most adequate study for dose-response modelling. The Panel also considered the study

performed by Epstein *et al.* (1969) in male Wistar rats (see Table 37). However, as all effective doses of AFB1 produced more than a 50% incidence of liver carcinomas this study was not found suitable for a BMD10 calculation, as this would be far outside the observable range for that study.

In the study by Wogan *et al.* (1974) groups of male Fisher rats, weighing approximately 80 g, were fed diets containing 0, 1, 5, 15, 50, or 100 µg/kg diet of AFB1 (purity >95%) until clinical deterioration of animals were observed, at which time all survivors in that treatment group were killed. The results of the study as regards liver pathology are given in Table 41. The Panel converted the dietary concentrations of AFB1 into daily intakes assuming that an average adult male rat consumed 40 g diet per kg body weight per day. The Panel also adjusted the daily intake to 104 weeks in order to compensate for the shorter study duration in some of the AFB1 groups. In the modelling of the results from the Wogan *et al.* (1974) study the highest dose was omitted because this dose resulted in a 100% tumour incidence.

Table 41: Induction of liver cell hyperplasia and tumours (hepatocellular carcinomas) in male Fisher rats after dietary administration of AFB1 (Wogan *et al.*, 1974).

Dose µg/kg b.w./day	Duration of dosing	Time adjusted dose	Tumour incidence	Hyperplasia	Transitional cells
0	104 w	0	0/18	1/18	0/18
0.04	104 w	0.040	2/22	6/22	1/22
0.2	93 w	0.179	1/22	4/22	1/22
0.6	96 w	0.554	4/21	13/21	0/21
2.0	82 w	1.58	20/25	8/25	7/25
4.0	54 w	2.1	28/28	8/28	4/28

The US EPA BMD software (BMDS) was used (US EPA, 2006) for modelling the liver carcinoma dose-response in male Fisher rats. For carcinogenicity data, a number of models are available in the BMDS, and model fitting, determination of goodness-of-fit, and comparing models to decide which one to use for obtaining the BMDL10 are outlined. The following dose-response models were fitted to the dose-incidence data:

- Gamma multihit model
- Log-logistic model
- Multistage model
- Probit model
- Quantal linear model
- Quantal quadratic model
- Weibull model

The BMD and BMDL values for an extra 10% risk compared to the background were estimated by performing 250 iterations.

The acceptability of a model can be based on several criteria. Some of the models are nested models (i.e. they are related to each other such that by leaving out a parameter, one model reduces to the other; this holds for the one, two, and three-stage models). The fit should not be significantly worse (using the likelihood ratio test) than the fit provided by the "full" model. The full model is the model that does not assume any dose–response function (its parameters are simply the frequencies per dose level) (Filipsson *et al.* 2003).

While the likelihood ratio test can only be applied to nested models, the AIC criterion (Akaike, 1974; Bozdogan, 1987) has been proposed as an approximate criterion for comparing the fits of non-nested models (Filipsson *et al.* 2003).

In addition, the BMDS provides statistics for the goodness of the fit. The lower the chi-square value the better the fit and the calculated p-value should be significantly larger than 0.1 which in this case was chosen to represent a rejection level (Filipsson *et al.* 2003).

For those models that were considered acceptable the BMD10 values, as well as the BMDL10 values were calculated (Table 42).

Table 42: BMD10 and BMDL10 calculation based on Wogan *et al.* (1974).

Model	Log (likelihood)	AIC	Chi-square	p-value	Accept	BMD10 (µg/kg b.w. per day)	BMDL10 (µg/kg b.w. per day)
Full model	-33.51						
Gamma multi-hit	-34.76	75.52	1.87	0.39	Yes	0.47	0.23
Log-logistic	-34.76	75.52	1.87	0.39	Yes	0.47	0.26
Multi-stage	-34.82	73.64	2.16	0.54	Yes	0.41	0.17
Probit	-33.75	75.50	1.80	0.41	Yes	0.48	0.28
Quantal-linear	-37.12	78.24	7.12	0.07	No		
Quantal-quadratic	-34.82	73.64	2.16	0.54	Yes	0.41	0.34
Weibull	-34.78	75.56	1.96	0.37	Yes	0.46	0.21

The calculated BMD10 values ranged from 0.41 to 0.48 µg/kg b.w. per day and the BMDL10 values from 0.17 to 0.34 µg/kg b.w. per day. In order to be prudent the Panel used the lowest BMDL10 of 0.17 µg/kg b.w. per day in the risk assessment.

Dose response modelling of human data

Assessment not accounting for HBV infection

Epidemiology data indicate a clear association between exposure to dietary aflatoxins and liver cancer. However the relationship is confounded by high incidences of hepatitis B, which is a recognised risk factor (see section 4.1.6.2). The potential carcinogenicity of

aflatoxins in humans has been examined in a number of population studies. Some of the studies do not differentiate between AFB1, AFG1, AFB2, and AFG2, but those included in the following (Table 43) provide separate evaluations of AFB1. The studies were carried out in Africa or Asia where substantial quantities of aflatoxins occur in basic foodstuffs, HBV infection is very common, and cancer of the liver is one of the most frequent forms of cancer in those regions. The studies are in the form of correlation studies, i.e. studies to determine whether there is a correlation between geographical differences in aflatoxin contamination of foodstuffs and the occurrence of liver cancer. The AFB1 intakes were calculated on the basis of measurements of the AFB1 content of foodstuffs (NFA, 1990).

Table 43: Population studies in four countries with a high incidence of liver cancer. HBV infection not taken into account (Adapted from NFA, 1990).

Country	Region	AFB1 intake (ng/kg b.w. per day)	Liver cancerRate/yeara)	Liver cancer rate/60 yearsa)
Kenya[b)]	Highland	4.2	14	840
Kenya	Midland	6.8	43	2,580
Kenya	Lowland	12.4	58	3,480
Swaziland[c)]	High veldt	14.3	35	2,100
Swaziland	Middle veldt	40.0	85	5,100
Swaziland	Lebombo	32.9	89	5,340
Swaziland	Low veldt	127.1	184	11,040
Transkei [d)]	Four districts	16.5	91	5,460
Mozambique	Manhica-Mangud	20.3	121	7,260
Mozambique	Massinga	38.6	93	5,580
Mozambique	Inhambane	77.7	218	13,080
Mozambique	Inharrime	86.9	178	10,680
Mozambique	Morrumbene	87.7	291	17,460
Mozambique	Homoine-Maxixe	131.4	479	28,740
Mozambique	Zavala	183.7	288	17,280
China[e)]	Guangxi B	11.7	1,754	105,240
China	Guangxi B	90.0	1,822	109,320
China	Guangxi C	704.5	2,855	171,300
China	Guangxi D	2,027.4	6,135	368,100

Age-adjusted annual incidence of liver cancer for men per one million individuals. The age distributions of the population groups studied did not deviate significantly from each other. In the study from China, the incidence of HBsAg+ carriers was 23% of all members of the cohort and in the study from Swaziland and (presumably) Kenya it was 21-28%, whereas no information was found for Mozambique. The calculation of the lifetime liver cancer rate (last column) assumed a lifespan of 60 years.

a) Peers *et al.*, 1976 as corrected by Carlborg, 1979.
b) Peers *et al.*, 1987.
c) Van Rensburg *et al.*, 1985.
d) Yeh *et al.*, 1989.

The Panel considered BMD analysis on the lifetime liver cancer rate data in Table 43 using the US EPA BMD software (BMDS) (US EPA, 2006). When the whole data set was used BMDL10 values for an extra 10% risk compared to the computed background risks ranging from 0.496 to 0.901 μg/kg b.w. per day were calculated. Due to the many data-points in the analysis the calculated BMD10 and BMDL10 values were very similar. However, the criteria for acceptability of the fits were not fulfilled in any case, and therefore the assessment was considered inappropriate. In addition, the Yeh *et al.* (1989) examined mortality from liver cancer, while the other studies also appeared to include diagnosis after clinical tests and histological examinations of needle biopsies. When the calculation was performed on the Yeh *et al.* (1989) data alone the range of BMDL10 values with acceptable fits were 870 – 1,100 ng/kg b.w. per day for an extra 10% risk compared to a computed background risk of about 10.5%. When the Yeh *et al.* (1989) data were removed from the data set BMDL10 values with acceptable fits were calculated from 344 to 862 ng/kg b.w. per day for an extra 10% risk compared to computed background risks of 0.17 – 0.50%, respectively. However, the Panel noted that the highest cancer incidence among these data was 2.8%, which is significantly lower than the BMR of 10% proposed by the EFSA Scientific Committee to be used as a point of departure for an assessment, and therefore this calculation was considered inappropriate. However, if the BMR was set to 1%, the data set without the Yeh *et al.* (1989) results could be used to calculate BMDL1 values with acceptable fits ranging from 78 – 121 ng/kg b.w. per day for an extra 1% risk compared to computed background risks of 0.17 – 0.50, respectively.

The JECFA (FAO/WHO, 1998) also reviewed selected risk assessments and compared the resulting potency estimates. The JECFA stressed, "that in all of the analyses, the potential effect of mis-specification of the dose that went into the derivation of the potency was not quantitatively addressed. As for all retrospective constructions of exposure, use of recent levels of aflatoxin exposure to describe current incidence rates assumes that current exposures are comparable to past exposures. Owing to the long latency period predicted for most cancers, uncertainty in the lifetime dose is an additional source of variability". The potency estimates based on the JECFA's analyses of epidemiological studies in which regional cancer rates were compared with estimates of

aflatoxin intake without regard to differences in HBV infection rates are summarise in Table 44.

The Panel noted that the potencies estimated by these various authors and cited by the JECFA were compatible with the BMDL values calculated by the Panel. As an example, a potency of 0.15 incidences/100,000 individuals per year from 1 ng AFB1/kg b.w. per day would correspond to an extra 1% or 10% lifetime (60 years) incidences from 111 or 1,111 ng/kg b.w. per day, respectively. This is, however, not surprising since the potencies were more or less derived by (linear) extrapolations from the same data that were used for the BMDL calculations.

Table 44: Potency estimates of the risk of liver cancer in humans based upon epidemiological data with no correction for HBV status assuming an exposure of 1 ng/kg per day (Adapted from the JECFA (FAO/WHO, 1998)).

Author	Incidence/year per 100 000[a)]
Peers & Linsell (1977)	0.11
Stoloff & Friedman (1976)	0
Carlborg (1979)	<0.21
Bruce (1990)	
based on Stoloff (1983)	0
based on van Rensburg *et al.* (1985), Shank *et al.* (1972a,b)	
Peers *et al.* (1976, 1987)	0.10
Croy and Crouch (1991)	
based on Peers *et al.* (1976)	0.15 (0.09, 0.23)
based on Yeh *et al.* (1989)	0.14 (0.08, 0.21)
Calif. Dept. Health Serv. (CDHS, 1990)	
based on Peers *et al.* (1976)	0.38 (0.15, 0.60)
based on van Rensburg *et al.* (1985)	0.14 (0.10, 0.17)
based on Peers *et al.* (1987)	0.17 (NA, 0.3)
based on Yeh *et al.* (1989)	0.18 (NA)

[a)] Numbers in parentheses represent (lower, upper) 95% confidence limits on the predicted risk when available from the authors.

Potency estimates accounting for HBV infection

The epidemiology study by Yeh *et al.* (1989) has been the focus of several quantitative risk assessments. The study has been considered useful in determining the potency of AFB1 exposure in $HBsAg^+$ individuals. It examined the roles of the hepatitis B virus and AFB1 in the development of primary HCC in a prospective cohort of 7,917 men aged 25

to 64 years old in the Guangxi Province in southern China, where the incidence of HCC is among the highest in the world. After accumulating 30,188 person-years of observation, 149 deaths were observed, 76 (51%) of which were due to HCC. Ninety-one per cent (69 of 76) were HBsAg+ at enrolment into the study in contrast to 23% of all members of the cohort. Three of the four patients who died of liver cirrhosis were also HBsAg+ at enrolment. There was no association between HBsAg positivity and other causes of death. To estimate AFB1 exposure, between 1978 and 1984, staple foods consumed in the counties of southern Guangxi were regularly sampled and tested for contamination by AFB1.

When estimated AFB1 levels in the subpopulations were plotted against the corresponding mortality rates of HCC, a positive and almost perfectly linear relationship was observed. On the other hand the prevalence of HBsAg was very high and homogeneous across the study areas (range 21.6%-24.7%) and therefore, no significant association was observed when the prevalence of HBsAg positivity in the subpopulations was compared with their corresponding rates of HCC mortality. The authors concluded that despite the "crudeness" of their exposure estimate, (i.e., population-based instead of personal exposure assessments), it is reasonable to conclude that AFB1 seems to play a role in the unusually high rates of HCC in southern Guangxi.

In the analysis of their study, Yeh *et al.* (1989) adjusted mortality rates for each region based on the age distribution of the composite study cohort as an internal standard. However, Wu-Williams *et al.* (1992) calculated that the age-adjusted HCC rate for the total cohort was 121.5 per 100,000 when standardized to the age distribution of the world population versus 226.3 per 100,000 when standardized to the age distribution of the study cohort. The ratio of these rates (0.54) was then used to adjust the regional HCC mortality rates reported by Yeh *et al.* (1989) to obtain expected incidence rates for a (hypothetical) cohort with age-distribution similar to the world population. Adjusted person-years of observation (APY) were calculated in each region as the number of HCC deaths observed in that region divided by the adjusted mortality rate. Adjusted person-years of observation were assumed to be distributed among HBsAg+ and HBsAg-carriers according to the regional prevalence of hepatitis B (FAO/WHO, 1998). These data are summarized in Table 45.

Table 45: Epidemiological data from Yeh *et al.* (1989) as corrected by Wu-Williams *et al.* (1992) (Adapted from the JECFA (FAO/WHO, 1998)).

AFB1 dose	PLC cases		APY[a]	
(ng/kg b.w./day)	$HBsAg^-$	$HBsAg^+$	$HBsAg^-$	$HBsAg^+$
12	0	12	9932	2727
90	1	7	6114	2017
705	4	12	7733	2537
2028	2	23	5803	1743
[b]	7	54	29582	9034

[a] Adjusted person-years (see text)
[b] No data available for this group

The data from the Yeh *et al.* (1989) study have been analysed by several authors in order to determine potencies of AFB1, expressed as cancers per 100 000 persons per year for every ng AFB1/kg b.w. per day, in HBV negative and HBV positive individuals exposed. Potencies obtained in these studies are summarized in Table 46.

Table 46: Potency estimates of the risk of liver cancer in humans based upon epidemiological data with correction for HBV status assuming an exposure of 1 ng/kg per day (Adapted from the JECFA (FAO/WHO, 1998)).

Study	HBsAg status	Incidence per 100,000[a]
Croy & Crouch (1991)	-	0.036 (0.079)
	+	0.50 (0.77)
Wu-Williams *et al.*(1992)		
Multiplicative-linear model	-	0.0037 (0.006)
	+	0.94 (0.19)
Additive-linear model	-	0.031 (0.06)
	+	0.43 (0.64)
Hosenyi (1992)	-	0.0018 (0.0032)
(background=3.4/100 000)	+	0.046 (0.08)
Bowers *et al.* (1993)	-	0.013
	+	0.328

[a] Numbers in parentheses represent upper 95% confidence limits on the predicted risk when available from the authors.

For its assessment, the JECFA chose separate central tendency estimated potencies and ranges for AFB1 from these epidemiological data. These corresponded to 0.3 cancers/year per 100,000 population per ng AFB1/kg b.w. per day (uncertainty range: 0.05-0.5) in hepatitis B virus antigen positive ($HBsAg^+$) individuals and 0.01 cancers/year per 100,000 population per ng AFB1/kg b.w. per day (uncertainty range: 0.002-0.03) in hepatitis B virus antigen negative ($HBsAg^-$) individuals (FAO/WHO, 1998).

Uncertainties in the human data used in the BMDL calculations and the potency estimates for AFB1

The Panel derived BMDL10 and BMDL1 values for the liver carcinogenicity of AFB1 based on epidemiological studies in populations with high incidences (>20%) of HBV infection. In order to take account of the importance of HBV infection in the liver carcinogenicity of AFB1 in human studies, the Panel decided to also use the JECFA potency estimates based on published assessments. However, the Panel recognised the large uncertainties behind all these estimates based on the human studies.

It should be noted that the association of aflatoxin exposure with increased liver cancer risk in HBV positive individuals is strong. It is rather the basis for the quantitative estimates of risk, related to AFB1 exposure and stratified by HBV status, which are uncertain. While the uncertainties related to the exposure estimates are similar in all the studies, the aflatoxin potency estimates comparing HBV positive and negative groups cited by the JECFA were predominantly derived from published analysis of the study by Yeh *et al.* (1989). Given the importance of this paper, the Panel wishes to mention a number of limitations and uncertainties that indicate that extrapolations from these data should be interpreted with caution.

Exposure uncertainties in the study of Yeh *et al.* (1989):

- Aflatoxin exposure was only calculated for the four agricultural communities in the study, as no information was available for the fifth community. The analysis was achieved by analysis of "raw" food samples from the counties of southern Guangxi collected twice a year between 1978 and 1984. As only raw food samples were analysed, it is unclear what effect sorting and food preparation might have had on aflatoxin levels in consumed foods. Analysis was restricted to AFB1 and was performed by thin-layer chromatography. There is no information on the type of foods sampled, the number sampled, the sampling plan, the size of each sample or the sub-sampling procedure; consequently the representative nature of the sample is unclear. Average (presumed to be mean) levels of AFB1 were used in calculating the aflatoxin intake from specific foods, however, the distribution of aflatoxin levels obtained to derive this average is not reported.
- Details on the laboratory methodology used, inclusion of controls, authentic standards, reproducibility, etc., may only be available from two Chinese language journals or Institute reports cited in the paper.
- An estimated mean intake per person was calculated by multiplying the yearly amount of a given food consumed by the population by the "average" AFB1 content to give total aflatoxin consumption from that food commodity. These values were summed for all staple foods and divided by the total population to give an estimated intake per person per year. Consumption of aflatoxins was divided equally among the population with no adjustment for age, sex etc. These levels were then correlated with mortality rates from liver cancer in the four communities.

- The estimated exposures (up to 2028 ng AFB1/kg b.w. per day according to the JECFA (FAO/WHO, 1998)) are higher than any other levels reported for other populations in sub-Saharan Africa or south-east Asia (IARC 1993). Therefore the estimates may be of limited relevance to lower exposure populations.
- The aflatoxin measures were almost at the same time period as the liver cancer mortality observations; past exposure may have been more relevant and different.

Other limitations:

- Only males were included in the cohort, between the ages of 25 and 64 years.
- The study examined mortality from liver cancer, rather than cancer incidence. The majority (67/76, 88%) of liver cancer deaths were diagnosed by raised serum alpha-fetoprotein and abnormalities on a liver scan; only two cases were diagnosed by histopathology.
- The completeness of cancer mortality data across communities is difficult to ascertain.
- The age-adjusted mortality rates (Table 43) were calculated on the basis of only between 8 and 25 liver cancer cases per community and the correlation between liver cancer rates and aflatoxin exposure was based on just four data points.
- HBV infection was investigated using a radioimmunoassay kit for HB surface antigen. The test was performed for all cases and for a random 25% of the cohort, stratified by age and county of residence. Twenty-two percent of this sub-cohort was positive for HBsAg compared to 91% of cases. There were only seven liver cancer cases in this study that were not HBsAg positive, however, as PCR-based technology was not available at the time of the study it is probable that some of these cases are false negatives for HBV infection.
- There was no adjustment for other confounders such as HCV, alcohol etc.,

Overall, the limitations in the aflatoxin exposure measurement, the exceptionally high exposure estimate, and the fact that the majority of liver cancer cases were found among HBV chronic carriers indicate that extrapolations from these data to populations with low exposures and low HBV status should be interpreted with caution.

It should also be noted that there is genetic variation in expression of genes which metabolize aflatoxin (Wild and Turner, 2002) and that the nature and prevalence of these differ among populations. There is therefore some uncertainty about the validity of potency estimates for aflatoxins being derived from one population and applied to others.

In order to use more recent epidemiological studies that have employed biomarkers (see Table 39) for risk assessment there would need to be a well-defined quantitative relationship between the biomarker and the aflatoxin exposure at the individual level. Although some studies have addressed this, these studies tend to be on a small scale, in one specific population, and the biomarker data are presented in categorical fashion (e.g. exposed/non-exposed; high exposure/low exposure) and hence the quantitative data on an individual basis are not available.

Nevertheless, the JECFA (FAO/WHO, 1998) used the data on urinary AFB1-N7-guanine adducts from Qian *et al.* (1994) for potency estimates, making a number of assumptions about the distribution of adduct levels, the average body weight of participants, daily urine volumes and percent excretion of AFB1 as AFB1-N7-Gua. A similar exercise was conducted for AFB-albumin adducts from the Wang *et al.*, (1996) study in a Taiwanese cohort. Despite a number of limitations, these calculations resulted in potency estimates in a similar range to those made from the modelling of the data from Yeh *et al.* (1989) by a number of authors.

5. Risk characterisation

The Panel evaluated whether the increase in dietary exposure to aflatoxins, predicted to result from altered regulatory MLs for almonds, hazelnuts and pistachios, would result in an increased risk based on the cancer potency estimates for AFB1 identified by the JECFA (FAO/WHO, 1998). Also, in line with the terms of reference and the opinions of the EFSA Scientific Committee and of the JECFA on substances that are genotoxic and carcinogenic (EFSA, 2005; FAO/WHO, 2005), Margins of Exposure (MOEs) were calculated by dividing the BMDL values for AFB1 derived from animal (rat) carcinogenicity and human epidemiological data by the estimates of dietary exposure. The Panel derived MOEs from the lowest BMDL10 (10% extra cancer risk) value of 170 ng/kg b.w. per day derived from the animal data and the lowest BMDL10 value of 870 ng/kg b.w. per day or the lowest BMDL1 (1% extra cancer risk) value of 78 ng/kg b.w. per day derived from epidemiological data. The EFSA Scientific Committee proposed that a MOE of 10,000 or higher, based on a BMDL10 from an animal study, would be of low concern from a public health point of view (EFSA, 2005). To date there have been no conclusions on the magnitude of an MOE based on human data that would be of low concern.

The exposure data taken from chapter 3.6 were calculated for total aflatoxins, whereas the dose response data are based on AFB1. Taking into account that AFB1 constituted a major proportion of total aflatoxins in the samples analysed, for the purposes of this evaluation, the Panel made the precautionary assumption that the potency of total aflatoxins is equivalent to that of AFB1.

5.1 Intake estimates and calculations of MOEs for the average EU population

The intake of aflatoxins from foods other than almonds, hazelnuts and pistachios was predominant in the estimates of population average intakes of aflatoxins. Applying the JECFA cancer potency estimates to the range of lower bound to upper bound estimates of mean exposure provides an indication of anticipated cancer incidence in different EU regions (Table 47). These take into account the lowest and highest reported prevalences of chronic HBV infection in the ranges reported by the WHO for Europe, which are 0.2% and 7%, respectively (see table 40, section 4.1.7.3). It is notable that the EU member

states with highest prevalence of HBV are in the Mediterranean region and also in GEMS/Food cluster B with the highest estimated aflatoxin intakes. These estimates are at least two orders of magnitude lower than the reported incidences of HCC in Europe, for which the age-standardised rate ranges from 1.7 – 11.6 cases/100,000 per year (chapter 4.1.7.1; Table 38), indicating that aflatoxins are unlikely to be a major contributor to HCC in the EU. This conclusion is supported by the observation that the specific mutation associated with aflatoxin exposure has a prevalence of less than 1% in Europe (chapter 4.1.7.2).

Table 47: Estimated cancer rates in different EU regions (data truncated at a ML of 4 µg/kg for almonds, hazelnuts and pistachios).

GEMS/ Food cluster	Total aflatoxin intake [a] Lower bound – upper bound (ng/kg b.w. per day)	Lowest HBV prevalence Cancers/yr per 100,000 [b]	Highest HBV prevalence Cancers/yr per 100,000 [c]
F	0.352 – 0.687	0.0037 – 0.0073	0.011 – 0.021
B	0.838 – 1.934	0.0089 – 0.0205	0.025 – 0.059

[a] Based on population average consumption and mean occurrence data taken from Table 26

[b] assuming 0.2 % have a risk of 0.3 cancers/year per 100,000, and 99.8% have a risk of 0.01 cancers/year per 100,000 per 1 ng/kg b.w. per day

[c] assuming 7 % have a risk of 0.3 cancers/year per 100,000, and 93% have a risk of 0.01 cancers/year per 100,000 per 1 ng/kg b.w. per day

MOEs calculated on the basis of the BMDL values from the animal and human data are shown in Table 48. The MOE based on the animal BMDL10 indicate a potential concern regarding aflatoxin intakes in all regions of the EU, even taking into account the uncertainty with respect to the large number of samples with aflatoxins below the LOD. However, the BMDL10 and BMDL1 values calculated based on human data from studies of sensitive populations (men only) having a high prevalence of HBV infection suggest that humans may be less sensitive than the rat strain used to derive the animal BMDL10.

Table 48: Estimated MOEs in different EU regions (data truncated at a MLs of 4 µg/kg for almonds, hazelnuts and pistachios).

GEMS/Food consumption cluster diets	Aflatoxins intake (ng/kg b.w. per day) [a]		MOE for animal BMDL10 [b]		MOE for human BMDL10 [c]		MOE for human BMDL1 [d]	
	LB	UB	LB	UB	LB	UB	LB	UB
F	0.352	0.687	483	247	2472	1266	222	114
B	0.838	1.934	203	88	1038	450	93	40

[a] Based on population average consumption and mean occurrence data based on data from table 26

[b] Rodent BMDL10 of 170 ng/kg b.w. per day divided by estimated lower bound-upper bound intake

[c] Human BMDL10 of 870 ng/kg b.w. per day, obtained from a study of a population (men only) with a high proportion of individuals being carriers of hepatitis B surface antigen and having a very high background incidence of hepatocellular carcinomas (app. 10%), divided by estimated lower bound-upper bound intake (see section 4.1.8)

[d] Human BMDL1 of 78 ng/kg b.w. per day, obtained from a study of a population (men only) with a high proportion of individuals being carriers of hepatitis B surface antigen and having a background incidence of hepatocellular carcinomas of <1%, divided by estimated lower bound-upper bound intake

LB = lower bound, UB = upper bound

The data in chapter 3.7 demonstrate that increasing the ML for total aflatoxins in almonds, hazelnuts and pistachios from 4 to 8 or 10 µg/ kg would increase total average dietary exposure by at most 1%, when calculated on the basis of mean occurrence data using either a lower bound or upper bound approach. Taking into account the uncertainties in the potency estimates, this would have a minimal effect on risk assessed at the population level. However, some subgroups could have higher intake due to their dietary habits, or be more susceptible to the effects of aflatoxins.

5.2 Vulnerable groups

High level consumers of nuts

The highest estimated aflatoxin intakes were derived for high level consumers of pistachios. These data are based on consumption data from a survey with a very low proportion of consumers and are therefore likely to overestimate long term exposure. The Panel used a worst case scenario, in which the upper end of the range of estimates for high level pistachio consumers, which were derived from limited consumption data, in a precautionary approach to assessing the risk to high level consumers. Table 49 shows the cancer risk estimates derived by applying the JECFA cancer potency estimates to the estimated intakes associated with MLs of 4, 8 and 10 µg/kg, again taking into account the lowest and highest reported prevalences of chronic HBV infection. These are slightly higher than the estimates for GEMS/food cluster B, in table 47 and show small increases

associated with increasing MLs, but are all still at least two orders of magnitude lower than the reported incidences of HCC in Europe.

Table 49: Estimated cancer rates for adult high level consumers of pistachios based on exposure estimates at different MLs for almonds, hazelnuts and pistachios.

Maximum total aflatoxin level (µg/kg)	Highest total aflatoxin intake [a] Lower bound – upper bound (ng/kg b.w. per day)	Lowest HBV prevalence Cancers/yr per 100,000 [b]	Highest HBV prevalence Cancers/yr per 100,000 [c]
4	0.980 – 2.251	0.010 – 0.024	0.030 – 0.068
8	1.115 – 2.386	0.012 – 0.025	0.034 – 0.072
10	1.187 – 2.450	0.013 – 0.026	0.036 – 0.074

[a] Based on high level consumer consumption and mean occurrence data
[b] assuming 0.2 % have a risk of 0.3 cancers/year per 100,000 per 1 ng/kg b.w. per day, and 99.8% have a risk of 0.01 cancers/year per 100,000 per 1 ng/kg b.w. per day
[c] assuming 7 % have a risk of 0.3 cancers/year per 100,000 per 1 ng/kg b.w. per day, and 93% have a risk of 0.01 cancers/year per 100,000 per 1 ng/kg b.w. per day

Similarly, the Panel used these worst case data in calculating MOEs (table 50). The MOEs are smaller than for the average population estimates in table 48, but show a minimal impact of changing the ML, regardless of whether the focus is on the lower bound or upper bound estimates. As noted in chapter 3.7, the greatest impact of changing the ML is predicted by using the lower bound estimates, but these over-estimate the impact because they are likely to under-estimate the exposure.

Children

The available data do not indicate that children have higher dietary exposure to aflatoxins than adults and therefore do not provide a basis for a different risk characterisation. However, the exposure estimates use the GEMS/Food data for dietary sources other than almonds, hazelnuts and pistachios, which are not specifically based of children's consumption patterns. Therefore this conclusion is tentative and better exposure data are required.

Table 50: Estimated MOEs for adult high level consumers of pistachios based on exposure estimates at different MLs for almonds, hazelnuts and pistachios.

Maximum total aflatoxin level (µg/kg)	Aflatoxin intake (ng/kg b.w.per day) [a]		MOE for animal BMDL10 [b]		MOE for human BMDL10 [c]		MOE for human BMDL1 [d]	
	LB	UB	LB	UB	LB	UB	LB	UB
4	0.980	2.251	173	76	888	386	80	35
8	1.115	2.386	152	71	780	365	70	33
10	1.187	2.450	143	69	733	355	66	32

[a] Based on high level consumer consumption and mean occurrence data

[b] Rodent BMDL10 of 170 ng/kg b.w. per day divided by estimated lower bound-upper bound intake

[c] Human BMDL10 of 870 ng/kg b.w. per day, obtained from a study of a population (men only) with a high proportion of individuals being carriers of hepatitis B surface antigen and having a very high background incidence of hepatocellular carcinomas (app. 10%), divided by estimated lower bound-upper bound intake

[d] Human BMDL1 of 78 ng/kg b.w. per day, obtained from a study of a population (men only) with a high proportion of individuals being carriers of hepatitis B surface antigen and having a background incidence of hepatocellular carcinomas of <1%, divided by estimated lower bound-upper bound intake

LB = lower bound, UB = upper bound

Vegetarians and vegans

The limited available exposure estimates for vegetarians and vegans are lower than for the highest national estimates for high level consumers of nuts. Therefore, these data also do not provide a basis for a different risk characterisation, but they are not directly comparable and again there is a need for better exposure data.

Subgroups with chronic hepatitis infection

Based on the JECFA potency estimates, the predicted cancer risk in subgroups with chronic hepatitis infection (i.e. subpopulation with 100% HBV infection) with the highest population average dietary exposure to total aflatoxins (cluster B) of 0.838 – 1.934 ng/kg b.w. per day (lower bound to upper bound) is 0.25 – 0.58 cancers per year per 100,000. These estimates are greater than for the general EU population (Table 47) where an HBV prevalence of 0.2-7% is assumed (see Table 40). However, no specific data are available for the HBV infected subgroup regarding consumption of nuts and other foods potentially contaminated with aflatoxins.

CONCLUSIONS

Exposure assessment

In total, 34,326 analytical results of aflatoxin occurrence in various food stuffs submitted by 20 Member States as well as 6,762 results from pre-export controls submitted by Turkey, each in response to a call for information by the European Commission, were considered for this assessment.

The Panel on Contaminants in the Food chain (CONTAM) also received data relating to concentrations of aflatoxin M1 in commercial milk samples. For almost all of these data, the values for aflatoxin M1 concentration were below 0.05 µg/kg and taking into account the lower carcinogenic potency of M1 the Panel did not consider these data further.

Aflatoxin contamination was found in many food commodities including maize, peanuts, dried fruit and spices, as well as tree nuts. Aflatoxin B1 was found to be the dominating aflatoxin in all foods. The highest total aflatoxin levels were found in pistachios and Brazil nuts. These two food commodities also showed the highest percentage of lots which did not comply with the current EU maximum levels.

The CONTAM Panel assessed data on almonds, hazelnuts and pistachios within the ranges of 0-4, 0-8 and 0-10 µg/kg of total aflatoxins in order to assess the potential impact of increasing the maximum levels.

Overall, 74% of all samples were below the limit of detection (LOD), which varied considerably between laboratories. For the statistical evaluation, the LOD was either entered as the actual numerical value (upper bound) or replaced by zero (lower bound).

A change in the current maximum level for **almonds** from 4 to 8 or 10 µg/kg total aflatoxins would add another 1.1% or 1.6% of lots as compliant and would result in an increase in the mean level for total aflatoxins from 0.40 to 0.46 or 0.50 µg/kg for upper bound and from 0.18 to 0.24 or 0.29 µg/kg for lower bound values.

A change in the current maximum level for **hazelnuts** from 4 to 8 or 10 µg/kg total aflatoxins would add another 2.7% or 3.9% of lots as compliant and would result in an increase in the mean level for total aflatoxins from 0.53 to 0.68 or 0.78 µg/kg for upper bound and from 0.31 to 0.46 or 0.57 µg/kg for lower bound values.

A change in the current maximum level for **pistachios** from 4 to 8 or 10 µg/kg total aflatoxins would add another 2.6% or 3.4% of lots as compliant and would result in an increase in the mean level for total aflatoxins from 0.44 to 0.61 or 0.69 µg/kg for upper bound and from 0.20 to 0.37 or 0.46 µg/kg for lower bound values.
In practice, it is unlikely that the controls are fully effective, and occasional consumption of nuts contaminated at the very much higher levels sometimes reported would further

increase the mean contaminant levels and hence reduce the relative impact of increasing the current maximum level from 4 to 8 or 10 µg/kg total aflatoxins in the three nuts.

Data on consumption of almonds, hazelnuts and pistachios were available for only a few Member States. Robust data on other sources of dietary exposure, representative of all Member States, were not available to the CONTAM Panel. Evaluation of the few available national dietary exposure data indicated that a reasonable approximation of European diets could be obtained from the GEMS/Food Consumption Cluster Diets database, and the CONTAM Panel therefore used these data in estimating dietary aflatoxin exposure from foods other than almonds, hazelnuts and pistachios.

The exposure assessment performed by the CONTAM Panel used a precautionary approach to the uncertainties with the result that the actual exposure is likely to be overestimated. The contribution from almonds, hazelnuts and pistachios was only a few percent of total dietary exposure to aflatoxins.

Increasing the maximum levels from 4 to 8 or 10 µg/kg would result in an increase in estimated average total dietary aflatoxin exposure in the region of 1%. Groups with high level nut consumption are more highly exposed and changing the permitted maximum levels for the three nut products could have an impact for some of these groups, with potential increases of up to 20% if the maximum levels were increased from 4 to 8 or 10 µg/kg and assuming a fully effective enforcement. If, as is expected, nuts exceeding the maximum levels are occasionally consumed, the total long term average dietary aflatoxin exposures might be higher, but the relative impact of raising the maximum level from 4 to 8 or 10 µg/kg in the three nuts would be less.

Estimated dietary exposures for children were within the range of estimates for adult populations, however these were predominated by exposure from foods other than nuts, for which data specific to children's diets were not available.

Hazard characterisation

Aflatoxin B1 is clearly genotoxic and carcinogenic in a variety of animal species. Increasing evidence demonstrates that aflatoxin B1 also has the potential to affect the immune system, nutrition and growth. The CONTAM Panel concluded that carcinogenicity is the critical effect on which to base the risk assessment. The carcinogenic potency of aflatoxins varied in different species and strains and in different studies. The CONTAM Panel used the most sensitive strain and sex in a precautionary approach to the risk characterisation. A BMDL10[9] of 170 ng/kg b.w. per day was calculated from a study involving administration of aflatoxin B1 at a range of dietary doses to male Fischer rats.

[9] 95% lower confidence limit of the benchmark dose for a 10 % increase in cancer incidence

A number of epidemiological studies have shown clear associations between aflatoxin exposure and incidence of hepatocellular carcinoma. These studies were performed in areas with high aflatoxin exposure and high prevalence of chronic hepatitis B, which is a recognised risk factor for liver cancer. Other factors possibly resulting in increased susceptibility are genetic and acquired variability in transport and metabolism of aflatoxins and also variability in DNA repair. There have been no direct investigations of the role of aflatoxin in the aetiology of hepatocellular carcinoma in Europe.

Although a role of aflatoxin in the aetiology of human hepatocellular carcinoma is generally accepted, using the human data as a basis for risk assessment is limited by the quality of aflatoxin exposure information and the low incidence of liver cancers in non-hepatitis B infected individuals in the available studies. The CONTAM Panel applied different approaches to the human data. It used the cancer potency estimates identified by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in its 1997 assessment which were mainly based on a single study from China, and derived a BMDL10 of 870 ng/kg b.w. per day from the same study, and a BMDL1[10] of 78 ng/kg b.w. per day from three studies from sub-Saharan Africa. As mentioned, all these studies used sensitive populations with a high prevalence of chronic hepatitis B and had very limited aflatoxin exposure information. In addition, the study from China had an exceptionally high exposure estimate and a very high (around 10%) frequency of hepatocellular carcinomas even in the lowest exposed group. These limitations increase the uncertainty in the accuracy of the estimates. Therefore, precise risk estimates cannot be derived for the European population.

Risk characterisation

Estimates of cancer risks based on potency estimates derived by the JECFA associated with the estimated average and high level dietary exposures to aflatoxins were at least two orders of magnitude lower than the reported incidences of hepatocellular carcinoma in Europe, suggesting that aflatoxins are unlikely to be a major contributor to hepatocellular carcinoma in the EU.

The margins of exposure (MOEs) calculated by the CONTAM Panel for all estimated intakes compared with the 95% lower confidence limit of the benchmark dose for a 10% increase in cancer incidence (BMDL10) based on animal data indicated a potential concern for human health. BMDL10 and BMDL1[11] values derived from data from human populations including the most sensitive subgroups with high prevalence of chronic hepatitis B infection, indicated similar sensitivity of this population to that of the most

[10] 95% lower confidence limit of the benchmark dose for a 1 % increase in cancer incidence

[11] 95% lower confidence limit of the benchmark dose for a 1 % increase in cancer incidence

sensitive strain of rat, but that other subgroups are likely to be less sensitive to the effects of aflatoxins.

The CONTAM Panel concluded that changing the maximum levels for total aflatoxins from 4 to 8 or 10 µg/kg in almonds, hazelnuts and pistachios would have minor effects on the estimates of dietary exposure, cancer risk and the calculated MOEs.

The CONTAM Panel concluded that exposure to aflatoxin from all sources should be as low as reasonably achievable, because aflatoxins are genotoxic and carcinogenic. The data indicate that reduction of total dietary exposure to aflatoxins could be achieved by reducing the number of highly contaminated foods reaching the market and reducing exposure from food sources other than almonds, hazelnuts and pistachios.

RECOMMENDATIONS

Occurrence

- There is a need for representative data for nuts and other foodstuffs, including total diet studies, to reduce uncertainties in the risk assessment. Methods should be applied that allow measurement of individual aflatoxins at concentrations well below the regulatory maximum levels.
- Measures to apply Good Agricultural Practice by producing countries are required in order to reduce incidents of highly contaminated products being consumed.
- Improved pre-export controls are also required to reduce incidents of highly contaminated products imported to the EU.
- Data on the efficiency of sorting process of nuts with different levels of aflatoxins are desirable.
- The possible aflatoxin contamination of foods grown in the EU should be kept under review, particularly in the light of potential changes in climate.

Exposure

- Exposure to aflatoxins from all food sources should be reassessed using harmonised national data collected in the EFSA food consumption database.
- A biomonitoring approach using validated biomarkers would complement food analysis and consumption data in providing information on prevalence and level of aflatoxin exposure in the EU.

Health effects

- Additional epidemiological studies examining the quantitative relationship between aflatoxin exposure, hepatitis B and C infection and liver cancer incidence are required to better perform quantitative risk assessment.
- Further investigations of the potential health implications of the effects of aflatoxins on the immune system and child growth are required.

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