DIPHENYLAMINE (030)

EXPLANATION

Diphenylamine was originally evaluated in 1969 and subsequently in 1976, 1979, 1984 and 1998. It was identified for re-evaluation within the CCPR Periodic Review Programme at the 1996 CCPR (ALINORM 97/24), proposed for consideration by the 2000 JMPR at the 1997 CCPR (ALINORM 97/ 24A) and the evaluation deferred until 2001. An ADI of 0-0.08 mg/kg bw was established by the 1998 JMPR replacing the previous ADI of 0-0.02 mg/kg bw.

Because of toxicological concerns, attention was given to the various method of synthesising and purifying diphenylamine to produce a quality acceptable for the treatment of apples and pears. It was proposed that a specification for a pure (food grade) diphenylamine should be drawn up.

The manufacturer reported new studies of physical and chemical properties, animal, plant and soil degradation, analytical methods, storage stability, farm animal feeding, supervised residue trials and food processing.

The governments of Australia and The Netherlands have reported information on national GAP and/or residue data.

IDENTITY

ISO common name: none

Chemical name

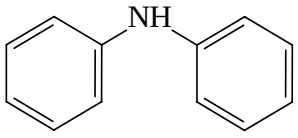
IUPAC: diphenylamine

CA: *N*-phenylbenzenamine

CAS Registry No.: 122-39-4

Synonyms: DPA

Structural formula:



Molecular formula: $C_{12}H_{11}N$

Molecular weight: 169.22

PHYSICAL AND CHEMICAL PROPERTIES

Pure active ingredient

Vapour pressure:	6.39×10^{-4} torr. (=8.52×10^{-2} Pa) at 25°C 2.32×10^{-3} torr. (=3.09×10^{-1} Pa) at 35°C 7.09×10^{-3} torr. (=9.46×10^{-1} Pa) at 45°C (gas saturation method) (Douglass, 1993a)	
Octanol/water partition coefficient:	K_{ow} = 3860, log K_{ow} = 3.6 at 25°C (batch method) (Douglass, 1993b)	
Solubility (99.4% purity):	water at 25°C acetonitrile at 25°C methanol at 25°C octanol at 25°C hexane at 25°C	0.039 mg/ml 860 mg/ml 474 mg/ml 230 mg/ml 57 mg/ml (Schetter, 1993)
Hydrolysis (sterile solution) (Baur, 1993):	half-life at 25°C	pH 5: 320 days pH 7: 350 days pH 9: 360 days (Baur, 1993)

Baur demonstrated that diphenylamine was substantially stable to hydrolysis under sterile conditions in the dark at 25°C for 30 days. The calculated half-lives are based on these 30-day tests.

Photolysis:	half-life 4.4 hours in aqueous buffer, pH 7, under a xenon arc lamp (157 W/m^2 (330-800 nm) (Baur and Robinson, 1993)
Dissociation constant:	pKa 1.03 at 20 ± 1.0°C (Hambrick, 1993)

Technical material

Appearance:	Cream solid flakes with a sharp creosote odour	(Wojcieck, 1992)
Relative density:	1.177 g/cm^3 at 25°C	(Wojcieck, 1992)
Melting point:	52.7-54.7°C	(Wojcieck, 1992)
Minimum purity:	>99%	
Solubility at 25ºC:	water acetonitrile methanol octanol hexane	0.038-0.042 mg/ml 808-897 mg/ml 454-492 mg/ml 204-237 mg/ml 53-66 mg/ml
Thermal stability:	25-150°C range without decomposition (Malone, 1993)	
Stability:	stable indefinitely	

FORMULATIONS

Diphenylamine is commercially available in EC and SL formulations.

METABOLISM AND ENVIRONMENTAL FATE

Animal metabolism

The Meeting received information on animal metabolism studies on rats, lactating goats and laying hens, all with diphenylamine uniformly labelled with ^{14}C in both rings of the molecule.

Rats. A metabolism study on diphenylamine (Wu, 1993) was evaluated by the JMPR in 1998 for the toxicological evaluation. Male and female Sprague-Dawley rats were treated orally with single doses of [^{14}C]diphenylamine at 5 and 750 mg/kg bw, and multiple doses of 5 mg/kg bw (repeated doses of 5 mg/kg bw/day of unlabelled diphenylamine for 14 days followed by single doses of 5 mg/kg bw of labelled compound). Diphenylamine was extensively absorbed and then excreted mainly in urine. Only 0.14-0.28% of the low dose remained in the tissues and organs of animals after 168 hours. ^{14}C in the expired air accounted for less than 0.01% of the administered dose. Twelve metabolites were identified. The parent and these metabolites in the excreta accounted for 81-93% of the dose. The metabolites were

4,4'-dihydroxydiphenylamine (4,4'-di-OH-DPA)	unconjugated
	O-sulfate
	O,*O*-disulfate
4-hydroxydiphenylamine (4-OH-DPA)	unconjugated
	O-glucuronide
	N-glucuronide
	O-sulfate
	O,*N*-diglucuronide
indophenol	unconjugated
	O-sulfate
3-hydroxydiphenylamine (3-OH-DPA)	unconjugated
2-hydroxydiphenylamine (2-OH-DPA)	unconjugated

Note: The hydroxy(di)phenylamines should be named as substituted phenols according to IUPAC nomenclature, but they are named as substituted phenylamines for clarity.

Goats. Two lactating goats were dosed orally with capsules containing [^{14}C]diphenylamine for seven successive days at a level equivalent to 45.5 ppm in the feed for Goat A (54 kg) and 46.6 ppm for Goat B (48 kg) (Kim-Kang, 1994c). Their diet consisted of a commercial grain ration and alfalfa mixed hay; average feed consumption per day was 2.38 kg and 1.94 kg respectively (moisture or dry weight content of the feed not stated). Milk was collected twice daily during the treatment period, and urine and faeces continuously throughout the treatment period. At the end of each 24-hour sampling each animal's cage was rinsed with isopropyl alcohol/water (IPA/H_2O, 1:1). The goats were slaughtered 24-26 hours after the last dose. Liver, kidneys, omental and back fat, tenderloin and hindquarter muscle were collected.

Approximately 97% of the administered dose was recovered from the urine, faeces, milk, cage wash, gastrointestinal tract contents and rumen contents (Table 1). Most of the radioactive residues were recovered in urine (>87%), and the total excretion through urine and faeces accounted for about 94%.

The total ^{14}C residues in the milk reached a plateau within a few hours. Because of rapid elimination after each daily administration the ^{14}C levels fluctuated regularly between the two milkings (Table 2). ^{14}C levels in the tissues were low, but higher in the kidneys and liver than in fat or muscle.

The levels of parent and metabolites identified in milk and tissues are shown in Table 3. 30-36% of the residues in fat and kidney remained as the unmetabolized parent compound, and 4-12% in the liver and milk. In addition to diphenylamine, several polar metabolites including 4-OH-DPA, 4,4'-dihydroxy-DPA, indophenol, the sulfate conjugate of 4-OH-DPA and the glucuronic acid conjugate of 4-OH-DPA were detected. A number of components of the residues in the liver including polar and non-polar unknowns were unidentifiable. Ring-hydroxylation followed by conjugation with either a sulfate moiety or glucuronic acid was the main metabolic pathway. In addition, hydroxylation on both rings followed by conjugation with either sulfate or glucuronic acid at only one position or at both positions were observed.

Table 1. Distribution of ^{14}C in 2 goats after dosing with [^{14}C]diphenylamine for seven days at a level equivalent to 45.5 or 46.6 ppm in the feed (Kim-Kang, 1994c).

	% of total ^{14}C dose						
	Urine	Faeces	Milk	Cage wash	GI tract contents	Rumen contents	Total recovery
Goat A	87.3	6.4	0.54	2.2	0.08	0.09	96.7
Goat B	89.1	5.5	0.56	1.4	0	0.05	96.6

Table 2. ^{14}C levels in milk of 2 goats during dosing at a level equivalent to 45.5 or 46.6 ppm in the feed for seven days and in the tissues after slaughter (Kim-Kang, 1994c).

Sample	Time (h)	^{14}C, mg/kg (as diphenylamine)	
		Goat A	Goat B
Milk	0	<0.01	<0.01
	8	0.78	0.53
	24	0.26	0.23
	32	0.89	0.64
	48	0.22	0.23
	56	0.79	0.63
	72	0.26	0.24
	80	0.77	0.66
	96	0.29	0.23
	104	0.86	0.66
	120	0.26	0.22
	128	0.75	0.62
	144	0.26	0.23
	152	0.85	0.63
	168 (7 days)	0.24	0.22
Liver	after slaughter	0.11	0.10
Kidneys	after slaughter	0.12	0.07
Leg muscle	after slaughter	0.007	0.006
Loin muscle	after slaughter	0.008	0.006
Back fat	after slaughter	0.026	0.02
Omental fat	after slaughter	0.02	0.02

Table 3. Parent compound and metabolites determined in the milk and tissues of goats dosed for 7 days with [^{14}C]diphenylamine (Kim-Kang, 1994c).

Compound	^{14}C expressed as diphenylamine, mg/kg							
	Milk [1]		Liver		Kidney		Omental fat	
	Goat A	Goat B	Goat A	Goat B	Goat A	Goat B	Goat A	Goat B
DPA	0.063	0.075	0.006	0.003	0.043	0.023	0.008	0.004
4-OH-DPA			0.002					
4,4'-di-OH-DPA			0.002	0.002				
Indophenol			0.002		0.002			
Metab A [2]	0.33	0.26	0.003		0.014	0.011		
Metab B [3]	0.40	0.25	0.009	0.006	0.029	0.018		
Metab I [4]	0.009		0.006		0.002			
Metab J [5]	0.013				0.002			
TOTAL [6]	0.85	0.63	0.11	0.10	0.12	0.07	0.02	0.02

[1] Milk: sampling time 152 hours
[2] Metab A: glucuronic acid conjugate of 4-OH-DPA
[3] Metab B: sulfate conjugate of 4-OH-DPA
[4] Metab I: sulfate conjugate of 4,4'-dihydroxy-DPA ?
[5] Metab J: disulfate conjugate of dihydroxy-DPA ?
[6] Total residue: ^{14}C measurement, see Table 2.

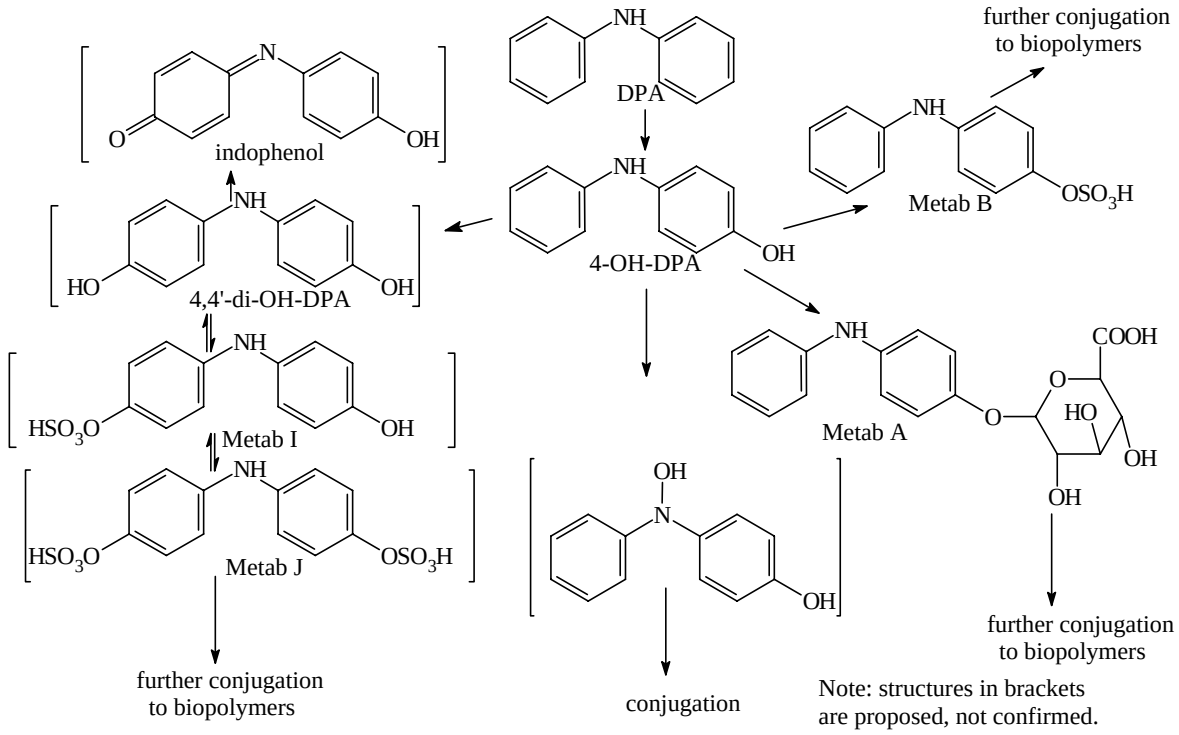


Figure 1. Proposed metabolic pathways of diphenylamine in the lactating goat.

Poultry. Fifteen laying hens were dosed orally with capsules containing [^{14}C]diphenylamine for seven days at levels equivalent to 50 ppm in feed, based on an average feed consumption (as fed, not expressed as dry weight) of 115 g/chicken/day (Kim-Kang, 1994d). Eggs were collected in the morning and in the afternoon and composited at each sampling for analysis. Hens were slaughtered approximately 22-24 hours after the last dose for analysis of liver, kidney, skin with adhering fat, and thigh and breast muscle.

Extraction and fractionation procedures were applied in conjunction with combustion, liquid scintillation counting (LSC), HPLC, and one- and two-dimensional TLC and radio-chromatography, to isolate and characterize significant metabolites. Preparative isolations of unknown metabolites were achieved by HPLC in order to obtain sufficient quantities for additional characterization by TLC. Two-dimensional TLC, in conjunction with base hydrolysis (sodium hydroxide), was employed to elucidate the structures of the aglycones of the conjugated metabolites.

Approximately 91% of the administered dose was excreted. The total ^{14}C as diphenylamine was 0.15 mg/kg in the liver, 0.21 in kidneys, <0.01 in breast and thigh muscle and 0.04 mg/kg in the fat/skin samples. The levels in the egg yolks ranged from <0.01 to 0.38 mg/kg (Table 4) and in the whites were less than 0.01 mg/kg.

Table 4. ^{14}C levels in eggs from 15 hens dosed at a level equivalent to 50 ppm in the feed during a seven-day study (Kim-Kang, 1994d).

Day	^{14}C, mg/kg (as diphenylamine)			
	Whites		Yolks	
	am	pm	am	pm
0		<0.01		<0.01
1	<0.01	<0.01	<0.01	<0.01
2	<0.01	<0.01	0.037	0.069
3	<0.01	<0.01	0.088	0.15
4	<0.01	<0.01	0.18	0.24
5	<0.01	<0.01	0.25	0.30
6	<0.01	<0.01	0.27	0.38
7	<0.01		0.31	

Table 5. Parent compound and metabolites in the eggs and tissues of hens dosed for 7 days with [^{14}C]diphenylamine at a level equivalent to 50 ppm in the feed (Kim-Kang, 1994d).

Compound	Metabolite levels, ^{14}C expressed as diphenylamine , mg/kg			
	Egg yolk [1]	Liver	Kidney	Fat + skin
DPA	0.065	0.011	0.003	0.014
2-OH-DPA		0.007	0.001	
4-OH-DPA	0.018			
4,4'-di-OH-DPA	0.002	0.004		
Indophenol		0.002		
Metab A [2]	0.012	0.002		
Metab B [3]	0.22	0.012		0.009
Metab C [4]	0.007	0.007	0.078	
Metab I [5]	0.005	0.003	0.020	
Metab J [6]	0.001		0.024	
TOTAL [7]	0.38	0.15	0.21	0.04

[1] Eggs: day 6 pm sample
[2] Metab A: glucuronic acid conjugate of 4-OH-DPA
[3] Metab B: sulfate conjugate of 4-OH-DPA
[4] Metab C: polar conjugate of 4-OH-DPA
[5] Metab I: sulfate conjugate of 4,4'-dihydroxy-DPA ?
[6] Metab J: disulfate conjugate of dihydroxy-DPA ?
[7] Total residue: ^{14}C measurement.

A significant proportion of the residues in the fat, skin and egg yolks remained as unmetabolized diphenylamine (7-35% of the ^{14}C) but most of the residue in the yolks was a sulfate conjugate of 4-OH-DPA (metabolite B, 57% of the ^{14}C). Minor residues in the yolks and liver were glucuronic conjugates of 4-OH-DPA. Some parent compound (1-8% of the ^{14}C) was also present in

the liver and kidneys. The main metabolite in the kidneys was a more polar conjugate of 4–OH-DPA (metabolite C, 38% of the ^{14}C). Only 32% of the ^{14}C in the liver was attributable to identified compounds (including metabolite C, Table 5) with two unidentified isomeric metabolites accounting for 48% of the residue.

The study of metabolism in hens demonstrated that diphenylamine can be hydroxylated on the ring at position 4 and subsequently conjugated with glucuronic acid, sulfate, and other groups, and also at position 2 to form 2-OH-DPA which can be conjugated subsequently with either a sulfate or glucuronic acid moiety. 4-OH-DPA can be further hydroxylated on the second ring to form dihydroxy-DPA, which can form either monoconjugates or diconjugates. Significant residues of diphenylamine and its degradation products formed by hydroxylation and conjugation with endogenous substrates were transferred into egg yolk and some tissues. Residues in egg white and muscles were below the limit of quantification.

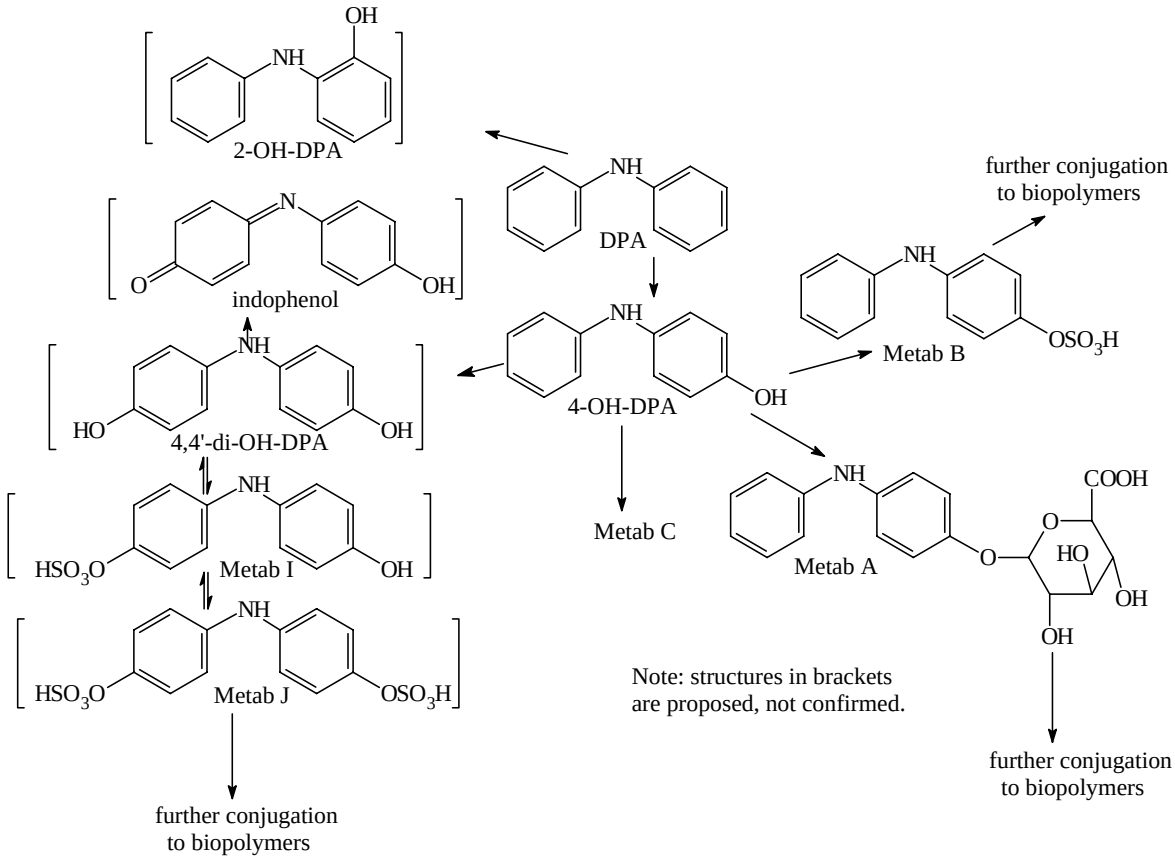


Figure 2. Proposed metabolic pathways of diphenylamine in laying hens.

Plant metabolism

A study of the metabolism of stored apples was reported to the Meeting (Kim-Kang, 1993, 1994a,b).

One hundred and forty-four Red Delicious apples, unit size about 200 g, were treated with an emulsion of [^{14}C]diphenylamine (uniform ring label). A residue of approximately 50 mg/kg resulted. All treated apples except eight used for zero-time measurement were stored in cabinets at 0 ± 2°C and 95 ± 5% relative humidity. Air was passed through the cabinets and an exit trap to collect volatile metabolites. Negligible amounts of the ^{14}C were found in the trap demonstrating that volatile

metabolites were not produced. Apples were sampled at intervals up to 40 weeks after treatment, with separation into peel and pulp for determination of the ^{14}C residues.

Most of the residue was absorbed into the peel within 2 days (Table 6), then it slowly migrated into the pulp which after 40 weeks contained 32% of the residue.

Under cold storage conditions the parent chemical is initially converted to a number of free hydroxylated products, including 2-hydroxydiphenylamine, 3-hydroxydiphenylamine, 4-hydroxydiphenylamine, and a dihydroxydiphenylamine (Table 7). The hydroxylated metabolites then conjugate with glucose and oligosaccharides. Diphenylamine is the main component of the residue in whole fruit.

Diphenylamine, in a simulation of commercial post-harvest treatment, was initially found on the surface of the apples. After 40 weeks' storage, the parent compound accounted for approximately 41% of the total ^{14}C residue with 37% present as conjugates and 8% as hydroxydiphenylamines. The proposed metabolic pathway is shown in Figure 3.

The unidentified non-polar metabolites found in the study were not related to either 4-aminobiphenyl, 2-aminobiphenyl, or *N*-nitrosodiphenylamine.

Table 6. Percentage distribution of the ^{14}C in methanol rinse, pulp and peel of apples treated post-harvest with [^{14}C]diphenylamine and stored at 0 ± 2°C and 95 ± 5% relative humidity(Kim-Kang, 1993).

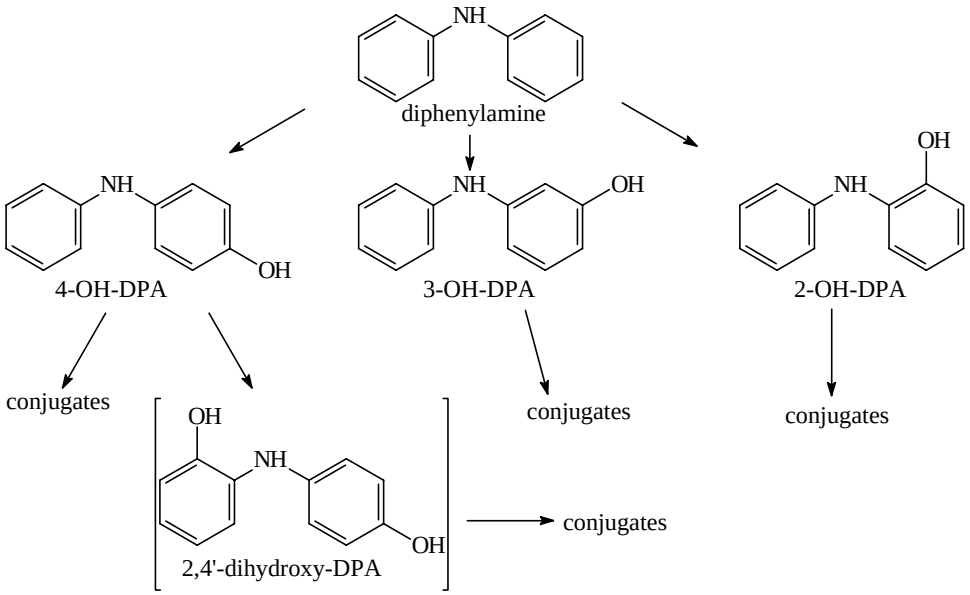
Days after treatment	% of the ^{14}C		
	Rinse (MeOH)	Pulp	Peel
0 (3.5 h)	77	0.13	23
2	29	3.0	68
7	22	5.6	73
14	23	7.4	69
28	21	9.9	69
42	16	14	69
56	16	17	67
69	15	19	66
84	14	15	71
112	15	22	63
140	11	22	67
168	10	26	65
196	10	31	59
224	8.2	27	64
252	9.3	28	62
280	7.0	32	61

Table 7. Distribution of diphenylamine and its metabolites in apples treated post-harvest with [^{14}C]diphenylamine and stored at 0 ± 2°C and 95 ± 5% relative humidity for 40 weeks (Kim-Kang, 1993).

Compound	Pulp		Peel		Rinse (MeOH)		Total fruit	
	% of TRR	mg/kg[1]	% of TRR	mg/kg[1]	% of TRR	mg/kg[1]	% of TRR	mg/kg[1]
Diphenylamine	12	2.0	50	76	87	2.4	41	17
2-OH-diphenylamine	0.51	0.09	1.6	2.4	1.7	0.05	1.3	0.54
3-OH-diphenylamine	1.0	0.16					0.33	0.14
4-OH-diphenylamine	3.5	0.57	7.2	11	7.7	0.21	6.1	2.6
Glucose conjugate of 4-OH-diphenylamine	21	3.4	13	19	1.3	0.04	14	6.1

Compound	Pulp		Peel		Rinse (MeOH)		Total fruit	
	% of TRR	mg/kg[1]	% of TRR	mg/kg[1]	% of TRR	mg/kg[1]	% of TRR	mg/kg[1]
Oligosaccharide conjugate of dihydroxy-diphenylamine	14	2.3	0.99	1.5			5.1	2.2
Oligosaccharide conjugate of 2-OH-diphenylamine	16	2.6	0.39	0.59			5.2	2.2
Oligosaccharide conjugate of 4-OH-diphenylamine	14	2.4	8.6	13			9.9	4.2
Oligosaccharide conjugate of 3-OH-diphenylamine	4.1	0.67	1.6	2.4	2.0	0.06	2.4	1.0
Polar unidentified	3.9	0.64					1.3	0.53
Polar unknowns 2 (9 metabs)	8.5	1.4	13	20			11	4.5
Nonpolar unknowns (2 metabs)	0.72	0.12	0.48	0.72			0.52	0.22
Bound residue	1.1	0.19	2.9	4.3			2.1	0.89
Total	100	16.5	100	151.1	100	2.76	100	42

[1] As diphenylamine



Conjugates: with glucose or oligosaccharides

Figure 3. Proposed metabolic pathways of diphenylamine in stored apples.

Environmental fate in soil

The Meeting received information on aerobic degradation, adsorption-desorption and mobility of aged residues.

Aerobic degradation. Liu (1993a) incubated ring-labelled [^{14}C]diphenylamine in a loam soil (45% sand, 36% silt, 19% clay, 0.6% organic matter, pH 7.3) at a nominal 10 mg/kg under aerobic conditions at 25°C in the dark for 12 months with soil moisture levels maintained at approximately 75% of field capacity. Recovery of ^{14}C, including volatiles, was in the range 91.4-103%.

The levels of diphenylamine, unextractables and $^{14}CO_2$ are shown in Table 8. Diphenylamine decreased rapidly in the initial stages, but more slowly after about 7 days. The products were polymeric and not identified. Mineralization was slow.

Table 8. Disappearance of diphenylamine and generation of CO_2 and unextractable residues during aerobic incubation of labelled diphenylamine in a loam soil (Liu, 1993a).

Day	Total ^{14}C	CO_2	Unextractable	diphenylamine
	^{14}C as % of applied			
0	101		4.5	91
4 h	103	0.01	12	83
8 h	103	0.06	22	42
1	100	0.30	26	54
3	101	1.4	48	31
7	99	2.3	53	21
14	99	3.7	53	18
30	99	5.3	57	9.9
60	97	7.9	46	21
91	94	10	49	11.3
120	99	11	55	15
184	101	14	58	21
270	91	17	48	18
365	98	18	49	15

Adsorption and desorption. Reynolds (1994) measured the adsorption and desorption of ring-labelled [^{14}C]diphenylamine on 4 soils and a sediment. Diphenylamine at 0.1-10 ng/g dissolved in 25 ml aqueous 0.01M $CaCl_2$ was added to 5 g of soil or sediment (the clay was tested at a soil:solution ratio of 1:100) and shaken at ambient temperature for 72 hours. ^{14}C was measured in clear solution removed after centrifugation; adsorption to the soil or sediment was calculated (Table 9). For the desorption test, 25 ml aqueous 0.01M $CaCl_2$ was added to the tube which was shaken for 72 hours at ambient temperature. ^{14}C was measured as above (Table 9). The stability of diphenylamine was tested by TLC analysis of the supernatants and residual desorption solids. The levels of diphenylamine accounted for 46-79% of the ^{14}C. According to the classification system based on K_{OC} diphenylamine was immobile (K_{OC} > 5000) in 2 soils, slightly mobile (K_{OC} 2000-5000) in 2 soils and of low mobility (K_{OC} 500-2000) in one soil.

Table 9. Adsorption and desorption of diphenylamine on 4 soils and a sediment (Reynolds, 1994).

Soil	Soil properties						Adsorption		Desorption		Mobility
	sand, %	silt, %	clay, %	organic matter, %	PH	CEC, meq/100 g	K_d	K_{oc}	K_d	K_{oc}	rating
Loam	45	36	19	0.60	7.3	9.6	13.8	3960	23.5	6750	slight
Silty clay loam, lake sediment	9.6	51	40	0.43	6.7	20	16.4	6590	40	15870	immobile
Clay	28	22	50	5.1	5.6	36	152	5140	307	10400	immobile
Loamy sand	78	16	6.0	1.0	6.6	9.0	21.4	3620	35	5860	slight
Silt loam	36	58	6.	0.7	7.8	14	4.9	1212	8.7	2150	low

Aged leaching. Kammerer (1994a) incubated ring-labelled [^{14}C]diphenylamine at 10 mg/kg in a loam, a loam sand, a silt loam and a clay soil under aerobic conditions in the dark at 25°C for 1 day and used the resulting aged residues for leaching experiments. On top of soil columns 30 cm high and 3.6 cm diameter were placed plugs (50 g) of the incubated soil, and the columns were then leached with 510 ml of 0.01M $CaCl_2$. Only 0.12-4.3% of the recovered ^{14}C appeared in the leachate, and 36-95% remained in the treated soil or the two top 6-cm segments (4.3-48% of that recovered) of the column. The mobility of aged diphenylamine in the 4 soils was loam, slight; loam sand and silt loam, low; and clay, immobile. Aged soils extracted with aqueous acetonitrile produced diphenylamine (14-56% of the dose) and the identified products *N,N*-diphenylformamide (2.0-4.7% of dose) and 4-nitro-*N*-phenyl-benzenamine (1.3-5.3% of dose).

Environmental fate in water-sediment systems

The Meeting received information on the photolysis of diphenylamine in aqueous solution and its anaerobic degradation in a sediment-water system.

Photolysis. Bauer (1993) UV-irradiated diphenylamine in an aqueous solution. Approximately 7% carbazole was formed in 0.5 hours peaking at 52% at 10.5 hours, hydroxydiphenylamine was about 1% after 1 hour and peaked at 16% by 36 hours, and D3, a cyclopentenohydroxyindole, reached 93% after 192 hours' irradiation. Minor amounts of trimeric products were also formed, with proposed structures based on molecular ions (M+1) at m/z 508, 515 and 534. Structures and proposed structures are shown in Figure 4.

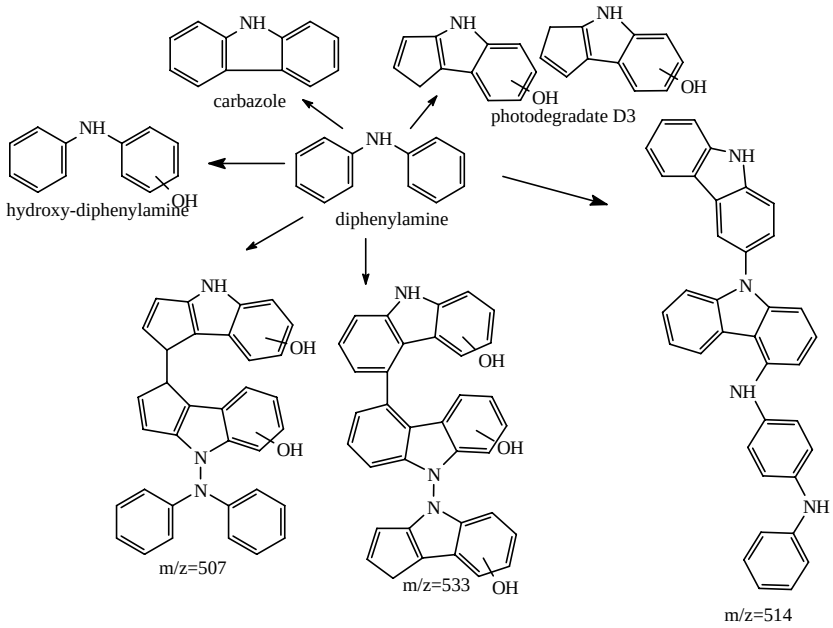


Figure 4. Photolytic degradation of diphenylamine.

Anaerobic aquatic degradation. Liu (1993b) incubated lake water (44 ml) and its sediment, a silty clay loam, (20 g dry weight or 26 g wet weight) with ring-labelled [^{14}C]diphenylamine at 10 mg/kg sediment in the dark at 25°C under anaerobic conditions for 1 year. Before incubation the water-sediment systems in the tubes had been flushed with nitrogen, dosed with 0.5 g glucose and incubated for 30 days to ensure anaerobicity. The lake water had a pH of 8.4 and an alkalinity of 112 mg/l expressed as $CaCO_3$. The sediment was the same as used in the adsorption-desorption experiments (Table 9). Evolved $^{14}CO_2$ was trapped. At sampling, sediment and water were extracted with acidified acetonitrile. ^{14}C recovery was acceptable, but was low at days 31 and 61 until it was realised that ^{14}C had been volatilized and incorporated into the rubber stoppers. Diphenylamine was identified as part

of the residue in the stoppers. The half-life of diphenylamine was approximately 60 days (Table 10). The degradation products were soil-bound or soil-incorporated residues, which very slowly mineralized.

Table 10. Residues of diphenylamine and its degradation products in lake sediment and water after anaerobic incubation at 25°C of ring-labelled [^{14}C]diphenylamine at 10 mg/kg sediment (Liu 1993b).

Days	Distribution of ^{14}C expressed as % of dose		
	$^{14}CO_2$	diphenylamine	unextractable
0		100	0.12
3	0.08	97	2.2
7	0.12	97	1.2
14	9.19	96	5.0
31	0.60	74	3.2
61	0.55	50	3.3
90	0.59	43	2.8
123	0.71	28	4.1
180	0.47	26	12
270	1.5	19	8.8
364	2.7	17	34

METHODS OF RESIDUE ANALYSIS

Analytical methods

The Meeting received information on methods of analysis for diphenylamine in crops, and for processed and animal commodities.

Apples. Tshabalala (1994a) described an analytical method for determining residues of diphenylamine in apples and their processed products.

Whole apples are homogenized in a food processor with liquid nitrogen to produce a white powder. An analytical sample is then blended with acetone and filtered. A portion of the filtrate is partitioned with hexane and water, from which the diphenylamine residue is recovered in the hexane extract, which is taken to dryness. The residue is taken up in dichloromethane for derivatization by reaction with trifluoroacetic anhydride reagent in dichloromethane in a sealed vial at 50°C for 1 hour. The acetylated diphenylamine is determined by GC-MS, and quantified using single-ion monitoring at 265 amu. Diphenylamine standards are also derivatized. The procedure for wet pomace, dry pomace and apple juice begins with the acetone extraction step. Recoveries are shown in Table 11.

The determined diphenylamine residues of 0.0686 and 0.085 mg/kg in control apples precluded reliable recovery tests at the lower levels. Tshabalala (1994b), in a supplementary report, showed that the LOQ for diphenylamine residues in apples, juice and wet pomace was 0.08 mg/kg, and in dry pomace 1 mg/kg.

Table 11. Recoveries of diphenylamine from apples and their processed commodities (Tshabalala, 1994a,b).

Sample	Fort. level, mg/kg	n	Mean recovery [1]	Recoveries [1]
Red Delicious whole apple	control	1	0.0686 mg/kg	0.0686 mg/kg
Red Delicious whole apple	0.02	3	125	96, 130, 150
Red Delicious whole apple	0.20	1	93	93
Red Delicious whole apple	2.0	5	91	110, 84, 88, 80, 95
Red Delicious whole apple	2.8	2	87	81, 92
Red Delicious juice	control	3	<0.08 mg/kg	<0.08, <0.08, <0.08 mg/kg
Red Delicious juice	0.40	3	83	62, 100, 86
Red Delicious juice	1.0	3	82	78, 80, 89

Sample	Fort. level, mg/kg	n	Mean recovery [1]	Recoveries [1]
Red Delicious juice	2.0	3	113	110, 120, 110
Red Delicious wet pomace	control	3	<0.08 mg/kg	<0.08, <0.08, <0.08 mg/kg
Red Delicious wet pomace	1.0	3	72	73, 70, 73
Red Delicious wet pomace	2.0	3	78	80, 78, 76
Red Delicious dry pomace	control	3	<0.40 mg/kg	<0.40, <0.40, <0.40 mg/kg
Red Delicious dry pomace	1.0	3	74	76, 70, 77
Red Delicious dry pomace	2.0	3	80	82, 79, 79
Granny Smith whole apple	control	3	0.085 mg/kg	0.094, 0.0803, 0.0802 mg/kg
Granny Smith whole apple	0.02	3	61	62, 67, 55
Granny Smith whole apple	0.20	3	80	86, 79, 75
Granny Smith whole apple	2.0	5	85	75, 78, 85, 96, 90
Granny Smith juice	control	3	<0.08 mg/kg	<0.08, <0.08, <0.08 mg/kg
Granny Smith juice	0.40	3	90	93, 90, 88
Granny Smith juice	1.0	3	91	91, 84, 99
Granny Smith juice	2.0	3	107	100, 110, 110
Granny Smith wet pomace	control	3	<0.086 mg/kg	<0.08, 0.099, <0.08 mg/kg
Granny Smith wet pomace	0.40	3	99	94, 110, 94
Granny Smith wet pomace	1.0	3	86	91, 76, 90
Granny Smith wet pomace	2.0	3	70	51, 86, 73
Granny Smith dry pomace	control	3	<0.08 mg/kg	<0.08, <0.08, <0.08 mg/kg
Granny Smith dry pomace	1.0	3	80	93, 78, 70
Granny Smith dry pomace	2.0	3	78	76, 74, 83
Supplementary report by Tshabalala, 1994b				
Red Delicious whole apple	control	1	<0.08 mg/kg	<0.08 mg/kg
Red Delicious whole apple	0.08	2	90	89, 90
Red Delicious whole apple	10.0	2	92	89, 95
Red Delicious juice	control	1	<0.08 mg/kg	<0.08 mg/kg
Red Delicious juice	0.08	1	69	69
Red Delicious juice	10.0	2	91	91, 90
Red Delicious wet pomace	control	1	<0.08 mg/kg	<0.08 mg/kg
Red Delicious wet pomace	0.08	2	83	83, 83
Granny Smith whole apple	control	1	<0.08 mg/kg	<0.08 mg/kg
Granny Smith whole apple	0.08	2	115	120, 110
Granny Smith whole apple	10.0	2	110	110, 110
Granny Smith juice	control	1	<0.08 mg/kg	<0.08 mg/kg
Granny Smith juice	0.08	2	93	93, 93
Granny Smith juice	10.0	2	87	81, 93
Granny Smith wet pomace	control	1	<0.08 mg/kg	<0.08 mg/kg
Granny Smith wet pomace	0.08	1	81	81

[1] % recovery for fortified samples, residue concentration for control samples

Thompson (2000) described the analytical method used in supervised trials on pears. The sample was extracted with acetone and cleaned up by repeated solvent partitioning. The residue in the final solution was analysed without derivatization by GLC with an NPD. The LOQ was 0.1 mg/kg. Recoveries were determined from 0.1-24 mg/kg and were generally low but adequate (mean 77%, range 58-93% n=20).

Animal tissues and milk. Keller and Weber (1996b) applied the enforcement analytical method for the determination of diphenylamine residues to samples of milk, liver, kidney and fat from a goat metabolism study (Kim-Kang, 1994c).

Diphenylamine is extracted from whole milk with acetonitrile, which is partitioned with hexane to remove fats. The acetonitrile extract is then evaporated to dryness, redissolved in hexane, and analysed by GC-MSD. The method for tissues is similar, except that after evaporation to dryness the residue is redissolved in a small volume of acetonitrile, diluted with water and partitioned into

hexane. The hexane solution is then analysed by GC-MSD. Recoveries are shown in Table 12. LOQs were 0.01 mg/kg.

Table 12. Recoveries of diphenylamine from goat milk and tissues (Keller and Weber, 1996b).

Sample	Fortification levels (mg/kg)	Mean % recovery	Recoveries, %
Milk	0.01	103	100, 105
Milk	0.10	93	80, 105
Kidney	0.01	111	101, 112, 106, 124
Kidney	0.025	97	97
Liver	0.01	106	100, 111
Fat	0.01	109	139, 80
Fat	0.025	131	131

Determinations by the analytical method are compared with ^{14}C measurements from the metabolism study in Table 13. The results are quite different for milk and kidney, but the measurements were made approximately 3 years apart and diphenylamine may have decreased during storage.

Table 13. Residues of diphenylamine in goat milk and tissues determined by the analytical method (Keller and Weber, 1996b) and by ^{14}C measurement (Kim-Kang, 1994c).

Sample	DPA by analysis (Nov 1995), mg/kg	DPA by ^{14}C measurement (Dec 1992), mg/kg
Milk	0.002	0.063
Kidney	0.002	0.043
Liver	0.004	0.006
Fat	0.006	0.008

Keller and Weber (1996a) used the same method for analysis of milk and tissues from a feeding trial on lactating dairy cows described later and provided validation data (Table 14). The LOQ was 0.01 mg/kg.

Table 14. Recoveries of diphenylamine from the milk and tissues of dairy cows (Keller and Weber, 1996a).

Sample	Fortification levels (mg/kg)	No.	Mean % recovery	Recoveries, %
Whole milk	0.01	3	98	105 94.9 93.9
Whole milk	1.0	3	91	90 91.6 91.6
Skimmed milk	0.01	3	85	103 90.5 61.8
Skimmed milk	1.0	3	88	89.7 85.9 87.9
Cream	0.01	3	112	105 115 115
Cream	1.0	3	96	94.1 98.6 94.6
Muscle	0.01	3	92	96.3 94.4 86.4
Muscle	1.0	3	97	111 74.8 106
Kidney	0.01	3	87	82.6 104 73.7
Kidney	1.0	3	93	100 85.6 91.9
Liver	0.01	3	83	86.5 90.5 71.7
Liver	0.10	3	104	109 104 99
Liver	0.30	3	95	104 96.4 84.2
Fat	0.01	3	95	95 106 85
Fat	0.10	3	94	71.5 106 103
Fat	0.20	3	94	95.5 96.5 90.1

Stability of residues in stored analytical samples

A trial on the stability of diphenylamine residues in fresh apples and processed commodities during frozen storage was reported to the Meeting (Johnson and Strickland, 1995a).

Red Delicious and Granny Smith apples were treated with a 0.20 kg ai/hl dip and 0.22 kg ai/hl drench respectively to simulate commercial post-harvest treatment and samples of whole apples, juice and pomace from the processed apple study described below (Johnson and Strickland, 1995c) were stored in freezers at temperatures between -24°C and -12°C. The results are shown in Table 15.

Degradation rates and the time required for 30% decrease of the residue were calculated from each test by assuming a first-order decrease (rate of decrease proportional to concentration). In each case the time required for 30% decrease exceeded the duration of the test (Table 15).

There were no measurable losses of diphenylamine residues in whole apple, juice or wet and dried pomace over the 155-202 days of the trial.

Table 15. Storage stability of diphenylamine in apples and their processed commodities (Johnson and Strickland, 1995a) Apples were dipped in diphenylamine solutions to simulate commercial post-harvest treatment and stored whole at -24°C to -12°C. Day zero samples from the processed apple residue study (Johnson and Strickland, 1995c) were used in the storage stability study on juice and pomace.

Commodity	Storage period days	DPA residues, mg/kg	Procedural analytical recoveries
Apple, Granny Smith, whole	7	4.3 4.2	
	21	4.4 4.8	
	41		75-86% (0.2-2 mg/kg)
	44	3.5 3.4	90% 96% (2 mg/kg)
	64	4.0 3.8	81% 88% (2 mg/kg)
	94	3.4 3.3	91% 95% (2 mg/kg)
	125	4.1 3.9	110% 110% (10 mg/kg)
	155	3.7 3.9	90% 110% (3.2 mg/kg)
Months for 30% decrease	>5		
Juice, Granny Smith	0	0.40 0.54	86% 94% (1 mg/kg)
	14	0.65 0.58	83% 94% (1 mg/kg)
	28	0.56 0.51	92% 98% (2 mg/kg)
	58	0.54 0.49	70% 86% (0.4 mg/kg)
	106	0.60 0.55	90% 97% (1 mg/kg)
	126	0.82 1.05	93% 110% (1.6 mg/kg)
	202	0.47 0.45	85% 90% (0.4 mg/kg)
Months for 30% decrease	>7		
Wet pomace, Granny Smith	0	98 97	73% 77% (200 mg/kg)
	14	105 88	80% 90% (200 mg/kg)
	28	124 105	89% 93% (200 mg/kg)
	58	117 102	92% 92% (120 mg/kg)
	91	96 106	86% 87% (120 mg/kg)
	122	107 120	110% 110% (120 mg/kg)
	161	92 85	79% 82% (120 mg/kg)
Months for 30% decrease	>6		
Dry pomace, Granny Smith	0	89 104	73% 77% (100 mg/kg)
	14	107 88	73% 87% (100 mg/kg)
	28	109 92	89% 88% (100 mg/kg)
	58	122 122	83% 88% (100 mg/kg)
	91	107 104	93% 100% (120 mg/kg)
	126	(101 108) [1]	64% 69% (100 mg/kg)
	167	86 91	77% 78% (100 mg/kg)

Commodity	Storage period days	DPA residues, mg/kg	Procedural analytical recoveries
Months for 30% decrease	>7		
Apple, Red Delicious, whole	7	2.6 2.5	80% 95% (2 mg/kg)
	12		81% 92% (2.8 mg/kg)
	21	2.9 3.2	93% 110% (0.2 2.0 mg/kg)
	44	3.6 3.6	84% 88% (2 mg/kg)
	64	4.7 4.8	87% 94% (2 mg/kg)
	94	3.6 3.9	94% 100% (2 mg/kg)
	125	2.8 3.2	89% 95% (10 mg/kg)
	155	3.4 3.7	87% 93% (3.2 mg/kg)
Months for 30% decrease	>5		
Juice, Red Delicious	0	0.80 0.76	96% 100% (2 mg/kg)
	14	0.78 0.95	85% 88% (2 mg/kg)
	28	0.80 0.87	85% 93% (2 mg/kg)
	58	0.81 0.86	83% 90% (0.4 mg/kg)
	106	0.90 0.87	98% 99% (1.6 mg/kg)
	126	0.65 0.78	77% 100% (1.6 mg/kg)
	202	0.62 0.62	86% 91% (0.4 mg/kg)
Months for 30% decrease	>7		
Wet pomace, Red Delicious	0	148 137	76% 98% (200 mg/kg)
	14	(117 110) [1]	67% 79% (200 mg/kg)
	28	147 126	86% 97% (200 mg/kg)
	58	132 151	82% 82% (160 mg/kg)
	91	146 134	99% 94% (160 mg/kg)
	122	126 155	79% 86% (120 mg/kg)
	161	137 131	81% 93% (160 mg/kg)
Months for 30% decrease	>6		
Dry pomace, Red Delicious	0	75 72	77% 91% (100 mg/kg)
	14	81 71	75% 74% (100 mg/kg)
	28	67 78	86% 98% (100 mg/kg)
	58	83 87	94% 99% (100 mg/kg)
	91	78 80	91% 97% (80 mg/kg)
	126	(62 59) [1]	59% 83% (100 mg/kg)
	167	72 78	77% 81% (100 mg/kg)
Months for 30% decrease	>6		

[1] Values in parentheses not taken into account because of poor procedural recoveries

In a storage stability study on animal products conducted as part of a livestock feeding study, whole milk, muscle and liver samples fortified at 0.101 mg/kg were stored at -20ºC ± 10ºC for 34 to 54 days. The results are shown in Table 16. During storage diphenylamine residues did not decrease significantly, but analytical variability may have camouflaged small losses.

Table 16. Stability of diphenylamine residues in milk, muscle and liver during freezer storage (Keller and Weber, 1996a).

Sample	Storage period (days)	Fort level, mg/kg	Residues mg/kg	% remaining	Procedural analytical recoveries
Whole milk	0	0.101	0.11, 0.11	109	
	14	0.101	0.090, 0.089	89	101% (0.101 mg/kg)
	31	0.101	0.095, 0.099	96	95% (0.101 mg/kg)
	54	0.101	0.093, 0.095	93	103% (0.101 mg/kg)
Muscle	0	0.101	0.100, 0.108	103	
	15	0.101	0.076, 0.076	75	85% (0.101 mg/kg)
	28	0.101	0.054, 0.062	57	66% (0.101 mg/kg)
	37	0.101	0.103, 0.098	100	111% (0.101 mg/kg)
Liver	0	0.101	0.114, 0.106	109	

Sample	Storage period (days)	Fort level, mg/kg	Residues mg/kg	% remaining	Procedural analytical recoveries
	15	0.101	0.096, 0.096	95	100% (0.101 mg/kg)
	28	0.101	0.101, 0.095	97	106% (0.101 mg/kg)
	38	0.101	0.105, 0.114	108	111% (0.101 mg/kg)

Definition of the residue

The plant metabolism study indicates that the parent compound diphenylamine is the main residue in apples.

The animal metabolism studies on rats, goats and laying hens indicate that the glucuronic and sulfate conjugates of 4-OH-DPA and the parent compound are the main components in animal tissues, milk and eggs.

The Meeting concluded that the current definition (diphenylamine only) is suitable for compliance with MRLs and for the estimation of dietary intake.

The measured log P_{ow} for diphenylamine is 3.6. The animal feeding study showed that diphenylamine residues in fat were higher than in muscle and in milk were associated with the cream, suggesting the compound should be designated fat-soluble. The Meeting recommended that diphenylamine should be described as fat-soluble.

USE PATTERN

Long-term exposure of apples to low temperatures in controlled-atmosphere storage commonly induces a physiological disorder known as scald. Diphenylamine is registered in a number of countries for post-harvest application to apples for reducing scald during storage. The two most commonly treated varieties are Red Delicious and Granny Smith. The information reported to the Meeting on registered uses is shown in Table 17.

Table 17. Registered uses of diphenylamine.

Crop	Country	Form	Application			Notes
			Method	Dip conc. kg ai/hl	Contact time	
Apples	Australia	EC 310 g/l	dip	0.05-0.36	minimum 10-30 secs	[1] [2]
Apples	France		dip	0.04-0.20	30 secs	[3]
Apples	France		drench	0.04-0.20	30 secs to 2 mins	[3]
Apples	Greece		dip, drench or fog	0.075-0.20	max 2 mins	[4]
Apples	Israel		drench or dip	0.20-0.30	max 2 mins	
Apples	Italy		dip, drench or fog	0.075-0.20	max 2 mins	[4]
Apples	Lebanon		dip, drench or fog	0.075-0.20	max 2 mins	[4]
Apples	South Africa		dip or spray	0.20	30 secs to 2 mins	general case
Apples	South Africa		dip or spray	0.20-0.30	30 secs to 2 mins	Granny Smith
Apples	Syria		dip, drench or fog	0.075-0.20	max 2 mins	[4]
Apples	Turkey		dip, drench or fog	0.075-0.20	max 2 mins	[4]
Apples	UK		dip	0.04-0.20	30 secs	[3]
Apples	UK		drench	0.04-0.20	30 secs to 2 mins	[3]
Apples	USA	EC 150 g/l	dip, spray or drench	0.10-0.22	max 2 mins	[5] [2]
Pears	Australia	EC 310 g/l	dip	0.037-0.26	minimum 10-30 secs	[1] [2]

Crop	Country	Form	Application			Notes
			Method	Dip conc. kg ai/hl	Contact time	
Pears	Greece		dip, drench or fog	0.075	max 2 mins	
Pears	Italy		dip, drench or fog	0.075	max 2 mins	
Pears	Lebanon		dip, drench or fog	0.075	max 2 mins	
Pears	Syria		dip, drench or fog	0.075	max 2 mins	
Pears	Turkey		dip, drench or fog	0.075	max 2 mins	

[1] Concentration depends on variety and intended storage. DPA moves into pears, thin-skinned and russet apples and pears faster than into thick-skinned apples such as Granny Smith. The rates for different varieties therefore vary.
[2] Label provided
[3] American red, Granny or Melrose : 1800-2000 ppm ; Idared, Jonagold, 2-colour variety : 900-1000 ppm ; Golden : 400-620 ppm
[4] Concentration depends on variety
[5] Use 0.10 kg ai/hl for Cortland McIntosh, Roma Beauty, Turley, Stayman and Winesap varieties; 0.20 kg ai/hl for Red Delicious and Fuji; 0.22 kg ai/hl for Granny Smith.

RESIDUES RESULTING FROM SUPERVISED TRIALS

Supervised trials on apples and pears were reported to the Meeting in full, with recoveries from fortified samples at appropriate levels and duration of sample storage. The results and dip concentrations have generally been rounded to 2 significant figures. The results are unadjusted for % recovery. Procedural recoveries for the Granny Smith and Delicious apples were mean 83% and 95%, SD 7.6% and 20% respectively.

In a trial from local orchards in the USA Red Delicious apples were dipped post-harvest and Granny Smith apples drenched, using duplicate treatment solutions and 75 apples of each kind weighing a minimum of 13 kg. Treated and control samples were placed in a controlled atmosphere and stored mainly at 0-1.5°C, 1.5% O_2 and 1.5-2% CO_2. The cold store for the Granny Smiths was opened on day 202 and until day 260 the temperature was approximately 5°C, and CO_2 and O_2 levels uncontrolled. Samples were withdrawn at intervals for analysis (Johnson and Strickland, 1995b). The results are shown in Table 18.

Pears from each of two local orchards in the USA were dipped. Four separate batches of diphenylamine dip solution were prepared and the pears (50 per batch) were dipped at the label rate for 2 min and set aside to dry (Thompson, 2000). Samples of 24 pears were then selected from each batch and stored in a freezer for 256 days. Three samples of pears spiked with diphenylamine at 9.6 mg/kg and stored in a freezer for 266 days retained 77-83% of the added diphenylamine. The results are shown in Table 19.

Table 18. Diphenylamine residues in apples from supervised trials with post-harvest treatments in WA, USA, 1993. Double-underlined residues are from treatments according to GAP and are valid for the estimation of an MRL. All EC formulations.

Variety	Application			Days[1]	Residues, mg/kg	Ref
	kg ai/hl	Type	No.			
Red Delicious	0.20	dip	1	0 90 181 281 0 90 181 261	<u>5.5</u> 4.6 4.3 3.5 c <0.08 (2) c <0.08 (2) c 0.16 0.13 c 0.15 0.21	DPA 93-01
Red Delicious	0.20	dip	1	0 90 181 281 0 90 181 261	6.2 <u>6.3</u> 3.6 2.9 c <0.08 (2) c <0.08 (2) c 0.16 0.13 c 0.15 0.21	DPA 93-01
Granny Smith	0.22	drench	1	0 91 181 260 0 91 175 240	<u>3.4</u> 2.5 2.0 1.1 c <0.08 (2) c 0.14 0.092 c 0.14 0.14 c 0.21 0.26	DPA 93-01
Granny Smith	0.22	drench	1	0 91 181 260 0 91 175 240	<u>3.4</u> 2.4 2.1 0.96 c <0.08 (2) c 0.14 0.092 c 0.14 0.14 c 0.21 0.26	DPA 93-01

[1] Treatment to sampling interval during controlled atmosphere storage
c: sample from untreated control

Table 19. Diphenylamine residues in Bartlett pears from supervised trials with post-harvest treatments in the USA in 2000 (Thompson, 2000). Double-underlined residues are from treatments according to GAP and are valid for MRL estimation. All EC formulations. All analyses on day of treatment.

Location	Application			Residues, mg/kg	Ref
	kg ai/hl	Type	No.		
ID	0.20	dip	1	2.1	06879.98-ID05
ID	0.20	dip	1	2.1	06879.98-ID05
ID	0.20	dip	1	2.9	06879.98-ID05
ID	0.20	dip	1	2.5	06879.98-ID05
WA	0.20	dip	1	2.3	06879.98-WA21
WA	0.20	dip	1	2.0	06879.98-WA21
WA	0.20	dip	1	2.4	06879.98-WA21
WA	0.20	dip	1	1.8	06879.98-WA21

Livestock feeding trials

In a feeding study reported to the Meeting (Keller and Weber, 1996a) lactating Holstein dairy cows (body weights ranging from 460-656 kg on the day before trial began) which had produced more than 19.3 kg of milk/day as an average during acclimatization, were given twice daily (after each milking) gelatine capsules containing diphenylamine for 28 days and slaughtered on day 29. Control animals were given empty gelatine capsules. Three groups each consisting of three cows were given doses equivalent to approximately 30, 90 and 300 ppm in the diet (dry-weight basis). Daily dry feed intake per animal ranged from 16.2 kg to 27.2 kg, mean 21.4 kg.

Samples of milk were collected on acclimatisation day 5 in the morning and then from each morning and afternoon milking from the day before the first dose until the morning of day 29. The animals were slaughtered within 22 hours of the last dose. Samples of liver, kidney, fat and muscle were homogenized and stored in a freezer for 1-54 days (milk), 34–35 days (muscle), 35-36 days (liver), 26-27 days (kidney), 19-20 days (fat) 12-15 days (cream) and 21-26 days (skimmed milk) before analysis. The results are shown in Table 21.

Levels of diphenylamine below the LOQ were detected in the milk on days 14, 21 and 24 in control samples, and at or about the LOQ (0.005 mg/kg) in the 30 and 90 ppm groups sometimes. Residues in milk from the 300 ppm feeding group were up to 0.014 mg/kg. When day 14 milk was separated into cream and skimmed milk residues partitioned into the fat fraction.

No residues were present in muscle at any dosage level and in kidneys only at the highest level. Residues were determined in fat, liver and cream at all feeding levels, allowing calculation of transfer factors (Table 20). Transfer factors for body fat and cream are quite similar, and in cream and liver appear to decrease with increased feeding levels.

Table 20. Transfer factors for diphenylamine in dairy cattle feed. Values are calculated for the mean and maximum residues in the 3 animals in the feeding group.

Feeding level, diphenylamine, ppm dry weight	Mean residue, mg/kg			Transfer factor = residue in sample ÷ feeding level		
	cream	liver	fat	cream	liver	fat
30	0.0098	0.034	0.006	0.00033	0.0011	0.00020
90	0.0190	0.053	0.0177	0.00021	0.00059	0.00020
300	0.0492	0.153	0.0533	0.00016	0.00051	0.00018
	Max residue, mg/kg					
		liver	fat	cream	liver	fat
30		0.068	0.006		0.0027	0.0002
90		0.070	0.020		0.00078	0.00022
300		0.257	0.109		0.00086	0.00036

Table 21. Residues in the milk and tissues of lactating Holstein dairy cattle, 3 animals per group, dosed twice daily for 28 days with diphenylamine equivalent to 30, 90 and 300 ppm in the diet on a dry-weight basis and slaughtered on day 29 (Keller and Weber, 1996a). Each recorded residue is from a single animal.

Sample	Diphenylamine, mg/kg [2]		
	Low dose (30 ppm)	Medium dose (90 ppm)	High dose (300 ppm)
Milk			
Day -1	<0.005, ND(2)	<0.005 (2), ND	<0.005, ND, <0.005
Day 1	<0.005 (3)	ND (2), <0.005	ND, <0.005, 0.005
Day 4	<0.005, ND, <0.005	<0.005 (3)	<0.005, 0.005, <0.005
Day 7	0.005 (3)	0.005, <0.005, 0.005	0.005, 0.006 (2)
Day 10	<0.005 (3)	<0.005 (3)	<0.005, 0.014, 0.008
Day 14 evening [1]	<0.005, 0.005, <0.005	0.006, 0.005, 0.006	<0.005, 0.006, 0.008
Day 14 morning	0.005, ND (2)	ND, 0.005 (2)	ND, 0.006, 0.005
Day 18	<0.005 (2), 0.005	<0.005 (3)	<0.005, 0.005 (2)
Day 21	0.005 (2), <0.005	0.005 (3)	0.006, 0.010, 0.008
Day 24	<0.005 (3)	<0.005, 0.005, <0.005	0.005, 0.012, 0.008
Day 28	<0.005 (3)	<0.005 (3)	<0.005, 0.005, <0.005
Skimmed milk			
Day 14 evening	ND (3)	ND (3)	ND (2), 0.005
Day 14 morning	<0.005, 0.011, ND	<0.005 (3)	<0.005 (3)
Cream			
Day 14 evening	0.011, 0.010, 0.008	0.028, 0.013, 0.020	0.014, 0.061, 0.059
Day 14 morning	0.009, 0.010, 0.011	0.017, 0.016, 0.020	0.013, 0.103, 0.045
Mean residue	0.0098	0.0190	0.0492
Liver			
	0.018, 0.016, 0.068	0.044, 0.045, 0.070	0.064, 0.257, 0.137
Mean residue	0.034	0.053	0.153
Kidney			
	<0.005 (3)	<0.005 (3)	0.006, 0.010, 0.006
Muscle			
	<0.005 (3)	<0.005 (3)	<0.005 (3)
Fat			
	0.006 (3)	0.020, 0.014, 0.019	0.022, 0.109, 0.029
Mean residue	0.006	0.0177	0.0533

[1] Note that "Day" refers to study day, which began when the first half-dose was given after the morning milking, so that the evening milking on study day 14 preceded the morning milking on study day 14.
[2] LOQ 0.01 mg/kg, LOD 0.005 mg/kg
ND: not detected

FATE OF RESIDUES IN STORAGE AND PROCESSING

Stability in commercial storage.

The Meeting received information on the stability of diphenylamine in whole apples after post-harvest treatments stored in controlled atmospheres at approximately 0-1°C, 1.2 -1.5% O_2 and 1.9% CO_2. The results are shown in Table 18.

Meherink *et al.* (1988) showed that diphenylamine residues on freshly-dipped Red Delicious apples at 20°C placed in a storage cabinet at 0°C were partially transferred to untreated pears already in the cabinet. After 30 days the levels of diphenylamine in the pears in the top and bottom of the container were 0.6 and 0.2 mg/kg respectively. It would appear that diphenylamine evaporated from the warm apples and condensed on the cold pears in storage.

Bramlage *et al.* (1996) investigated whether diphenylamine is endogenous in apples. Hexane rinses from the surface of freshly harvested apples from a number of sources, when examined by GC-MS, contained a diphenylamine-like substance. It may have been diphenylamine contaminated with a similar compound or a related compound that after derivatization with heptafluorobutyric anhydride produced three mass spectral ions at 168, 278 and 365 as in derivatized diphenylamine, but with different abundances. The authors concluded that the presence of endogenous diphenylamine was not proved but in any case its concentration would not exceed 0.01 mg/kg.

In processing

The Meeting received information on the fate of diphenylamine during commercial processing of apples (Johnson and Strickland, 1995c).

Red Delicious apples were dipped in 2.0 kg ai/hl diphenylamine and Granny Smith apples drenched at 2.2 kg ai/hl, both treatments at 10 times the US label rate. Including controls, all treatments included the fungicide thiabendazole at 0.053 kg ai/hl to prevent spoilage in storage, which is standard industrial practice. Some treated and control apples were processed directly while others were placed in a commercial controlled atmosphere store before processing. Six samples of both varieties were treated; the day 0 samples each consisted of 150 apples (approximately 27 kg), and the stored samples 75 apples (approximately 13 kg). Duplication in the experiment was based on duplicated treatment solutions.

Apples were processed into juice, wet pomace and dried pomace using simulated small-scale industrial procedures. The apples were washed in a tub, sorted, crushed, and pressed to wet pomace which was dried in a bin air dryer at 79-93°C to less than 10% moisture to produce dried pomace. The juice was filtered to produce a fresh juice fraction. The results and calculated processing factors are shown in Table 22.

Calculated processing factors from unwashed apples to processed products were juice, mean 0.051, range 0.022-0.12; wet pomace, mean 4.7, range 2.3-8.4; dry pomace, mean 2.4, range 1.4-3.6. Diphenylamine is volatilized during drying resulting in a lower processing factor for dry than for wet pomace.

Table 22. Diphenylamine residues in apples, juice and pomace from processing treated apples after treatment or after controlled atmosphere storage.

Apple variety	Commodity	Apple storage period, days [1]	Diphenylamine, mg/kg	Processing factor [2]
Red Delicious	unwashed apples	0	33.6	
	juice	0	1.27	0.038
	wet pomace	0	128	3.8
	dried pomace	0	63.5	1.9
Red Delicious	unwashed apples	0	25.6	
	juice	0	1.48	0.058
	wet pomace	0	160	6.3
	dried pomace	0	46.2	1.8
Red Delicious	unwashed apples			
	juice	181	0.93	
	wet pomace	181	74.1	
	dried pomace	181	52.5	
Red Delicious	unwashed apples			
	juice	181	1.10	
	wet pomace	181	92.8	
	dried pomace	181	55.6	
Red Delicious	unwashed apples	281	28.8	
	juice	281	0.85	0.030
	wet pomace	281	66.7	2.3
	dried pomace	281	40.4	1.4
Red Delicious	unwashed apples	281	10.3	
	juice	281	0.66	0.064
	wet pomace	281	62.4	6.1
	dried pomace	281	34.0	3.3
Granny Smith	unwashed apples	0	31.6	
	juice	0	0.694	0.022
	wet pomace	0	99.0	3.1
	dried pomace	0	99.8	3.2
Granny Smith	unwashed apples	0	25.3	
	juice	0	0.628	0.025
	wet pomace	0	89.5	3.5
	dried pomace	0	38.3	1.5
Granny Smith	unwashed apples			
	juice	181	0.910	
	wet pomace	181	72.6	
	dried pomace	181	35.6	
Granny Smith	unwashed apples			
	juice	181	0.494	
	wet pomace	181	51.9	
	dried pomace	181	15.0	
Granny Smith	unwashed apples	260	4.63	
	juice	260	0.558	0.121
	wet pomace	260	38.8	8.4
	dried pomace	260	16.5	3.6
Granny Smith	unwashed apples	260	5.68	
	juice	260	0.284	0.050
	wet pomace	260	23.4	4.1
	dried pomace	260	13.8	2.4

[1] Controlled atmosphere

[2] Processing factor: residue in processed commodity ÷ residue in unwashed apples

RESIDUES IN FOOD IN COMMERCE OR CONSUMPTION

Monitoring data

The government of The Netherlands reported data for diphenylamine on several commodities to the Meeting. The results are summarized in Table 23.

Table 23. Monitoring data for diphenylamine on several commodities in The Netherlands.

Commodity	No. of samples	No. of samples with residues [1]	MRL, mg/kg	No. of samples >MRL	Year
Apples	1495	57	5		1994-1996
Apples	398	10	5		1997
Apples	93	5	5		1998
Asparagus	244	0	0.05*		1994-1996
Aubergines	148	1	0.05*	1	1994-1996
Bananas	57	0	0.05*		1994-1996
Beans (with pods)	133	1	0.05*	1	1998
Beetroot	83	0	0.05*		1994-1996
Carrots	407	0	0.05*		1994-1996
Courgettes	206	0	0.05*		1994-1996
Cucumbers	951	0	0.05*		1994-1996
Endive	1137	0	0.05*		1994-1996
Grapes	667	0	0.05*		1994-1996
Iceberg lettuce	471	0	0.05*		1994-1996
Lettuce	3306	0	0.05*		1994-1996
Mangoes	191	0	0.05*		1994-1996
Mushrooms	384	0	0.05*		1994-1996
Onion	97	0	0.05*		1994-1996
Oranges	902	0	0.05*		1994-1996
Passion fruit	40	0	0.05*		1994-1996
Pears	34	3	0.05*	3	1998
Peppers	1525	0	0.05*		1994-1996
Plums	437	0	0.05*		1994-1996
Pulses	42	0	0.05*		1994-1996
Strawberries	2378	1	0.05*	1	1994-1996
Swedes	9	0	0.05*		1994-1996
Sweet corn	27	0	0.05*		1994-1996
Tomatoes	1108	0	0.05*		1994-1996

[1] Limit of quantification 0.05 mg/kg

Monitoring data from the USDA Pesticide Data Program for 1994, 1995, 1996 and 1997 are summarized in Table 24.

Table 24. Monitoring data from the USDA Pesticide Data Program.

Commodity	No. of samples	No. of samples with residues	Number of samples in residue range (mg/kg)							Year
			<0.05	<0.5	<1.0	<2.0	<3.0	<5.0	>5.0	
Apple juice	171	17	14	3						1996
Apple juice	668	57	45	12						1997
Apples	629	438	76	117	125	93	16	10	1	1994
Apples	691	489	73	138	118	131	21	7	1	1995
Apples	524	452	52	121	146	105	22	6	0	1996
Bananas	508	0								1994
Bananas	479	1		1						1995
Broccoli	545	0								1994
Carrots	554	0								1994
Carrots	701	0								1995
Carrots	500	0								1996

Commodity	No. of samples	No. of samples with residues	Number of samples in residue range (mg/kg)							Year
			<0.05	<0.5	<1.0	<2.0	<3.0	<5.0	>5.0	
Celery	143	0								1994
Grapes	537	1	1							1994
Grapes	677	1	1							1995
Grapes	519	0								1996
Green beans	473	0								1994
Green beans	587	0								1995
Green beans	525	0								1996
Green beans	693	6	5	1						1997
Lettuce	542	0								1994
Milk	346	0								1996
Orange juice	678	2	2							1997
Oranges	506	0								1994
Oranges	549	0								1995
Oranges	454	1		1						1996
Peaches	299	0								1994
Peaches	285	1	1							1995
Peaches	735	1		1						1997
Peaches	280	5	2	3						1996
Potatoes	539	0								1994
Potatoes	692	1		1						1995
Spinach	477	0								1995
Spinach	441	0								1996
Spinach	498	0								1997
Spinach, canned	168	2	1	1						1997
Squash	426	1	1							1997
Sweet corn	327	0								1994
Sweet corn	671	0								1995
Sweet corn	173	0								1996
Sweet peas	245	0								1994
Sweet peas	601	0								1995
Sweet peas	355	0								1996
Sweet potatoes	507	1	1							1996
Sweet potatoes	695	1	1							1997
Tomatoes	168	0								1996
Tomatoes	707	4	4							1997

Monitoring data from a review of the US Food and Drug Administration Pesticide Monitoring Database for Fiscal years 1992, 1993, 1994, 1995, 1996, 1997 and 1998 are shown in Table 25.

Table 25. US FDA pesticide monitoring data for diphenylamine.

Commodity	No. of samples with residues	Number of samples in residue range (mg/kg)							Year
		<0.05	<0.5	<1.0	<2.0	<3.0	<5.0	>5.0	
Apples	45			4	4	16	15	6	1992
Apples	8		2	1	2	3			1993
Apples	6	1	1	2	2				1994
Apples	16	5	3	2	4	2			1995
Apples	28	13	15						1996
Apples	18	4	6	4	1	2	1		1997
Apples	31	15	10	2	4	1			1998
Apple juice	1			1					1993
Apple juice	1		1						1994
Apple juice	2	2							1996
Apple juice	5	5							1997

Commodity	No. of samples with residues	Number of samples in residue range (mg/kg)							Year
		<0.05	<0.5	<1.0	<2.0	<3.0	<5.0	>5.0	
Apple juice	1	1							1998
Pear	6	2	4						1995
Pear	1	1							1996
Pear	5	2	3						1997
Pear	4	3	1						1998

Data for diphenylamine residues in pears from the targeted monitoring program in Victoria, Australia, were reported for 1995-97. The results are shown in Table 26.

Table 26. Diphenylamine residues in pears from the targeted monitoring program from Victoria, Australia, for 1995-97.

No. of samples analysed	No. of samples with diphenylamine	No. of samples in range (mg/kg)						
		<0.1	<0.5	<1.0	<2.0	<3.0	<4.0	<5.0
26	26	0	7	4	7	3	3	2

Diphenylamine was included in the 1996 Australian Market Basket Survey (Hardy, 1998). The estimated daily dietary intake for diphenylamine residues in food expressed as % ADI for diets based on mean energy intake were 2.1% for adult males, 2.8% for adult females, 3.2% for boys aged 12, 2.6% for girls aged 12, 11.8% for toddlers and 8.6% for infants (ADI 0.02 mg/kg bw).

NATIONAL MAXIMUM RESIDUE LIMITS

The Meeting was informed of the following national MRLs.

Country	MRL, mg/kg	Commodity
Australia	5	Apples
	7	Pears
USA	10	Apples
	0	Meat, milk
Netherlands	5	Apples
	0.05*	Other food commodities

APPRAISAL

Diphenylamine was first evaluated in 1969. Its toxicology was reviewed by the 1998 JMPR, which allocated an ADI of 0–0.08 mg/kg bw and concluded that an acute RfD was unnecessary. Diphenylamine was reviewed by the present Meeting within the CCPR periodic review programme.

The Meeting received information on physical and chemical properties, metabolism and environmental fate, analytical methods, stability on storage, farm animal feeding, use pattern, residues in supervised trials on apples and pears and a study of processing.

METABOLISM

Animals

When rats were dosed orally with [^{14}C-ring]diphenylamine at 5 or 750 mg/kg bw, the compound was extensively absorbed and was excreted mainly in urine. Only 0.14–0.28% of the dose remained in tissues and organs of animals at the low dose 168 h after dosing. The radiolabel in expired air accounted for < 0.01% of the administered dose. Twelve metabolites were identified, most of which were hydroxylated diphenylamines and their glucuronide and sulfate conjugates. The parent and these 12 metabolites accounted for 81–93% of the dose in excreta.

When two lactating goats were dosed orally with encapsulated [^{14}C]diphenylamine for 7 days at a level equivalent to 46 ppm in the feed, the total amount excreted in urine and faeces accounted for about 94% of the dose. TRRs in milk reached equilibrium rapidly. The concentrations of radiolabel in tissues were low, but more was found in kidney and liver than in fat or muscle. A significant proportion of the residues in fat and kidney (30–36%) and some residues in liver and milk (4–12%) were unmetabolized parent compound.

Several polar metabolites were identified, including 4-hydroxydiphenylamine, 4,4'-dihydroxy-diphenylamine, indophenol and the sulfate and glucuronic acid conjugates of 4-hydroxydiphenylamine. Ring hydroxylation followed by conjugation with either a sulfate moiety or glucuronic acid was the main metabolic pathway.

Approximately 91% of the administered dose was recovered in the excreta of 15 laying hens dosed orally with encapsulated [^{14}C]diphenylamine for 7 successive days at a level equivalent to 50 ppm in feed. The concentrations of TRR (as diphenylamine) in liver, kidneys, breast muscle, thigh muscle and fat/skin samples were 0.15, 0.21, < 0.01 < 0.01, and 0.04 mg/kg, respectively, while those in egg yolk ranged from < 0.01 to 0.38 mg/kg and those in egg whites were < 0.01 mg/kg. A significant proportion of the residues in fat and skin and egg yolk were unmetabolized parent compound (17–35%); however, most (58%) of the residues in egg yolk were identified as a sulfate conjugate of 4-hydroxydiphenylamine, which also appeared in the other tissues.

The study of metabolism in hens showed that diphenylamine can be hydroxylated on the ring at position 4 or 2 and hydroxylated on the second ring. All these metabolites may subsequently be conjugated with glucuronic acid, sulfate and other groups.

PLANTS

In a study of the metabolism of diphenylamine in stored Red Delicious apple, the fruit were treated with an emulsion of [U-^{14}C-ring]diphenylamine, resulting in a residue of approximately 50 mg/kg, and stored at 2 °C and 95% relative humidity for 40 weeks. Most of the pesticide was absorbed into the peel within 2 days. The residue then slowly migrated into the pulp, and, after 40 weeks, the pulp contained 32% of the residue. After 40 weeks' storage, the parent compound accounted for approximately 41% of the TRR in the apples, with 37% as conjugates and 8% as hydroxydiphenylamines. Diphenylamine was converted to 2-, 3- and 4-hydroxydiphenylamines and a dihydroxydiphenylamine, which was then conjugated with glucose and oligosaccharides. The unknown non-polar metabolites, accounting for 0.52% of the residue in the apples, were not related to 4-aminobiphenyl, 2-aminobiphenyl or *N*-nitrosodiphenylamine.

ENVIRONMENTAL FATE

SOIL

When [^{14}C-ring]diphenylamine was incubated in a loam soil at a nominal rate of 10 mg/kg under aerobic conditions at 25 °C in the dark for 12 months, diphenylamine initially disappeared rapidly, but after about 7 days the disappearance was quite slow. After 12 months 15% of the dose remained as diphenylamine, 49% was unextractable, and 18% was mineralized. The metabolites were polymeric and not identified.

When [^{14}C-ring]diphenylamine was tested for adsorption and desorption on four soils and a sediment, its mobility ratings were slight, immobile, immobile, slight and low in a loam, a silty clay loam lake sediment, a clay, a loamy sand and a silt loam, respectively.

Residues of [^{14}C-ring]diphenylamine were aerobically aged on four soils for 1 day and then leached through columns of the soils with 0.01 mol/L $CaCl_2$. The mobility ratings for the aged residues were: loam, slight; loam sand, low; silt loam, low; clay, immobile. Metabolites were identified in extracts from the soil columns as *N*,*N*-diphenylformamide (2.0–4.7% of dose) and 4-nitro-*N*-phenylbenzenamine (1.3–5.3% of dose).

WATER–SEDIMENT SYSTEMS

In a photolysis study, carbazole was identified as a major product when diphenylamine in an aqueous solution was subjected to UV irradiation, with approximately 7% formed within 0.5 h and a maximum of 52% at 10.5 h. Hydroxydiphenylamine was also identified, reaching a maximum value of 16% by 36 h. A third product, an indenohydroxyindole, reached a value of 93% by the end of the 192-h irradiation period. Small amounts of trimeric products were also formed.

When [^{14}C-ring]diphenylamine was incubated with a lake water and sediment under anaerobic conditions in the dark at 25 °C, the half-life for disappearance was approximately 60 days. The products of decomposition were soil-bound or soil-incorporated residues, which mineralized slowly (2.7% of the dose in 1 year).

METHODS OF ANALYSIS

The Meeting received information on GLC methods for the analysis of diphenylamine residues in fruit, processed apples and animal commodities.

Plant matrices

Whole apples were homogenized in a food processor with liquid nitrogen to produce a white powder, and an analytical sample of homogenized whole apples or wet pomace, dry pomace or apple juice was blended with acetone and filtered. Diphenylamine residues were extracted from the aqueous acetone

with hexane and then derivatized with trifluoroacetic anhydride in dichloromethane to produce trifluoroacetylated diphenylamine for GLC–MS analysis. The LOQ for diphenylamine residues in apples, juice and wet pomace was 0.08 mg/kg, and that for dry pomace was 1 mg/kg. The mean recovery from the four matrices was 85% (range, 51–150%, *n* = 85).

In the analytical method used in a supervised trial on pears, the sample was extracted with acetone and subjected to a number of solvent partitioning clean-up steps. The residue in the final solution was analysed, without derivatization, by GLC–NPD. The LOQ was 0.1 mg/kg. Recoveries were tested after addition of 0.1–24 mg/kg and were generally low, but satisfactory (mean, 77%; range, 58–93%; *n* = 20).

Animal matrices

In the method of analysis for diphenylamine residues in milk and animal tissues, the matrix was extracted with acetonitrile, which was partitioned with hexane to remove fats. The acetonitrile extract was then evaporated to dryness, redissolved in hexane, and analysed by GLC with mass-selective detection. The LOQ was 0.01 mg/kg. In validation testing for whole milk, skim milk, cream, muscle, kidney, liver and fat after spiking with 0.01–1 mg/kg, the mean recovery was 94% (range, 62–115%; *n* = 96).

Tissues and milk from goats in the study of metabolism were analysed for diphenylamine for comparison with the measurement of [^{14}C]diphenylamine. The results were reasonably close for liver and fat, but not for milk and kidney. However, the measurements were made approximately 3 years apart, and diphenylamine may have depleted during storage.

STABILITY OF RESIDUES IN STORED ANALYTICAL SAMPLES

Data on stability during freezer storage were provided for diphenylamine residues in whole apples, juice, wet pomace, dry pomace, whole milk, muscle and liver. The residues in whole apples, juice, wet pomace, and dry pomace were stable for 5–7 months, those in whole milk for 8 weeks and those in muscle and liver for 6 weeks.

DEFINITION OF THE RESIDUE

The parent compound diphenylamine is the main component of the residue in apples. The gluconic and sulfate conjugates of 4-hydroxydiphenylamine and the parent compound are the main components in animal tissues, milk and eggs. The conjugates of 4-hydroxydiphenylamine can be regarded as intermediates in the process of detoxication and excretion and need not be included in the residue definition for dietary risk assessment. All the plant metabolites were also animal metabolites.

The Meeting concluded that the current definition (diphenylamine only) is suitable for assessing compliance with MRLs and for estimating dietary intake.

The measured log P_{ow} for diphenylamine is 3.6. The animal feeding study showed that the concentrations of diphenylamine residue in fat were higher than in muscle, and that in milk diphenylamine was associated with the cream, suggesting the compound should be designated fat-soluble. The Meeting recommended that diphenylamine be described as fat-soluble.

RESULTS OF SUPERVISED TRIALS

Diphenylamine is registered for post-harvest use on apple in the USA as a dip, spray or drench at a concentration of 0.20 kg ai/hl for Red Delicious and 0.22 kg ai/hl for Granny Smith and a maximum contact time of 2 min. The concentrations of residues in apples in four trials meeting GAP in the USA were 3.4, 3.4, 5.5 and 6.3 mg/kg.

Although data were available on residues from only four trials, the Meeting agreed that the data were sufficient because post-harvest trials need not cover the range of variables that occur in a field situation. The trials did include dip and drench methods of application.

The Meeting estimated a maximum residue level and an STMR value for diphenylamine in apples of 10 and 4.45 mg/kg respectively. The estimated maximum residue level replaces the current recommendation (5 mg/kg) for apple.

Diphenylamine is registered for post-harvest use on pears in Australia as a dip at a concentration of 0.037–0.26 kg ai/hl and a minimum contact time of 10–30 s. The concentrations of residues in pears in eight trials in the USA that matched Australian GAP (The dip concentration of 0.20 kg ai/hl was 23% below specified GAP, but sufficiently close.), in rank order (median underlined), were: 1.8, 2.0, 2.1 (2), 2.3, 2.4, 2.5 and 2.9 mg/kg.

The Meeting agreed that Australian GAP could be applied to the trials in the USA for post-harvest use. The Meeting estimated a maximum residue level and an STMR value for diphenylamine in pears of 5 and 2.2 mg/kg, respectively.

FATE OF RESIDUES DURING STORAGE AND PROCESSING

Treated apples from the supervised trials were held in commercial cold storage, and diphenylamine residues were measured at intervals. The concentrations declined with an average half-life of 7–8 months. There is some evidence that small amounts of diphenylamine may be transferred from treated to untreated fruit in the same store.

Fate of residues during processing

When diphenylamine-treated apples were processed into juice, wet pomace and dried pomace by procedures that simulated small-scale industrial practices, the residues tended to concentrate in the pomace and deplete in the juice. The first step in the process was washing, which would be expected to reduce surface residues.

The calculated processing factors for unwashed apples to processed commodity were: juice, mean 0.051, range 0.022–0.12; wet pomace, mean 4.7, range 2.3–8.4; dry pomace, mean 2.4, range 1.4–3.6. Diphenylamine is volatilized during drying, resulting in a lower processing factor for dry pomace than for wet pomace.

The Meeting applied these processing factors to the estimated maximum residue level (10 mg/kg) and STMR value (4.45 mg/kg) for apples to provide estimates for the processed commodities. The Meeting estimated a maximum residue level and an STMR-P value for diphenylamine in apple juice of 0.5 and 0.23 mg/kg respectively, an STMR-P value for diphenylamine in wet apple pomace of 21 mg/kg, and a maximum residue level and an STMR-P value for diphenylamine in dry apple pomace of 25 and 10.6 mg/kg, respectively.

RESIDUES IN ANIMAL COMMODITIES

DIETARY BURDEN IN FARM ANIMALS

The Meeting estimated the dietary burden of diphenylamine residues in farm animals on the basis of the diets listed in Appendix IX of the *FAO Manual*. As the only feed commodities listed are processed, the dietary burdens for the estimated MRL and STMR value are the same.

Commodity	Group	Residue (mg/kg)	Basis	Dry matter (%)	Residue, dry weight (mg/kg)	Choose diets (%)			Residue contribution (mg/kg)		
						Beef cattle	Dairy cows	Poultry	Beef cattle	Dairy cows	Poultry
Apple pomace, wet	AB	21	STMR-P	40	53	40	20		21	11.5	
Apple pomace, dry	AB	10.6	STMR-P	90	11.8						
					Total	40	20		21	11.5	

FEEDING STUDIES

Groups of three lactating Holstein dairy cows were dosed orally by capsule twice daily for 28 days (once after each milking) at a dose equivalent to 30, 90 and 300 ppm in the diet (dry weight). The animals were slaughtered on day 29 for tissue collection and analysis.

Diphenylamine residues were detected in milk on some occasions in the groups given 30 or 90 ppm, but at or about the LOQ (0.005 mg/kg). The concentrations of residues in milk from cows at 300 ppm were up to 0.014 mg/kg. When milk collected on day 14 was separated into cream and skim milk, the residues partitioned into the fat fraction.

The concentration of residues in muscle was < 0.005 mg/kg, even at the highest feeding level, and those in kidney were just measurable (0.006–0.01 mg/kg) at this level. Residues were measured in liver, fat and day-14 cream in cows at all three feeding levels, the mean values being 0.034, 0.053 and 0.153 mg/kg in liver; 0.006, 0.0177 and 0.053 mg/kg in fat; and 0.0098, 0.019 and 0.0492 mg/kg in

cream at the three feeding levels. The transfer factor (residue in tissue ÷ residue in feed) for fat was consistent across feeding levels: 0.00020, 0.00020 and 0.00018, but the factors for cream and liver were less consistent.

MAXIMUM RESIDUE LEVELS

The dietary burdens of diphenylamine for estimation of MRL and STMR values in animal commodities (residue concentrations in animal feeds expressed in dry weight) were 21 mg/kg for beef cattle and 11.5 mg/kg for dairy cows. As the dietary burden for beef cattle is higher than that for dairy cows, it should be used to estimate residues in tissues. The dietary burdens were lower than the lowest feeding level (30 ppm), so the resulting residues in tissues and milk were calculated by applying the transfer factors at the lowest feeding level to those dietary burdens.

The highest individual concentration of tissue residue in the relevant feeding group was used in conjunction with the dietary burden to calculate the probable highest residue in animal commodities. The mean value in tissues from animals in the relevant feeding group was used in conjunction with the dietary burden to estimate the STMR values for animal commodities. For milk (cream), the mean residue in milk (cream) at the plateau level in the relevant feeding group was used to estimate both the highest residue and the STMR value.

Feeding level (ppm) *Interpolated* / Actual	Diphenylamine residues (mg/kg)				
	Cream	Fat		Liver	
	(mean)	High	Mean	High	Mean
MRL beef cattle *21* / 30		*0.0042* / 0.006		*0.048* / 0.068	
MRL dairy cows *11.5* / 30	*0.0038* / 0.0098				
STMR beef cattle *21* / 30			*0.0042* / 0.006		*0.024* / 0.034
STMR dairy cows *11.5* / 30	*0.0038* / 0.0098				

The concentrations of residues in muscle and kidney were < 0.005 mg/kg and 0.007 mg/kg, respectively, at the highest feeding level (300 ppm). The Meeting agreed that residues in muscle and kidney at a feeding level of 21 mg/kg were unlikely to exceed 0.0005 and 0.0007 mg/kg, respectively.

The Meeting estimated a maximum residue level and an STMR value for diphenylamine in cattle meat of 0.01* (fat) and 0.0005 mg/kg, respectively; a maximum residue level and an STMR value for diphenylamine in cattle liver of 0.05 and 0.024 mg/kg, respectively; a maximum residue level and an STMR value for diphenylamine in cream of 0.01* and 0.0038 mg/kg, respectively, which are equivalent to 0.0004* F and 0.00015 mg/kg for milk; and a maximum residue level and an STMR value for diphenylamine in cattle kidney of 0.01* and 0.0007 mg/kg, respectively.

Recommendations

On the basis of the available data on residues resulting from supervised trials, the Meeting estimated the maximum residue levels and STMR values listed below. The maximum residue levels are recommended for use as MRLs.

Definition of residue (for compliance with the MRL and for estimation of dietary intake): Diphenylamine. The residue is fat-soluble.

Commodity		MRL (mg/kg)		STMR/STMR-P (mg/kg)
CCN	Name	New	Current	
FP 0226	Apple	10 Po	5 Po	4.45
JF 0226	Apple juice	0.5 PoP		0.23
AB 0226	Apple pomace (dry)	25 PoP		10.6
	Apple pomace (wet)			21
MO 1280	Cattle, kidney	0.01*		0.0007
MO 1281	Cattle, liver	0.05		0.024
ML 0812	Cattle milk	0.0004* F[1]		0.00015
MM 0812	Cattle meat	0.01* (fat)		0.0005
FP 0230	Pear	5 Po		2.2

* At or about the LOQ
[1] Equivalent to 0.01* mg/kg in milk fat.

Dietary risk assessment

Long-term intake

The periodic review of diphenylamine resulted in recommendations for new and revised MRLs and new STMR values for raw and processed commodities. Data on consumption were available for the food commodities and were used in calculating dietary intake. The results are shown in Annex 3.

The IEDIs for the five GEMS/Food regional diets, based on estimated STMRs, were 0–4% of the ADI. The Meeting concluded that long-term intake of residues of diphenylamine from uses that have been considered by the JMPR is unlikely to present a public health concern.

Short-term intake

The 1998 JMPR concluded that an acute RfD for diphenylamine was unnecessary. The Meeting therefore concluded that the short-term dietary intake of diphenylamine residues is unlikely to present a risk to consumers.

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