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Research Article

ENVIRONMENTAL CHEMICAL CONTAMINANTS

Determination of Carcinogenic Primary Aromatic Amines Contained as Impurities in Synthetic Organic Colorants

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Abstract

Background: Several primary aromatic amines (PAAs) have been designated carcinogenic or suspected of carcinogenicity. Several kinds of PAAs may occur either via the reduction of azo compounds or as impurities in azo colorants or other agents.
Objective: An analytical method was developed and applied to determine whether certain PAAs are present as impurities in synthetic organic colorants.
Method: Target chemicals were analyzed by the ultrasound extraction of the synthetic organic colorant with a hydrochloric acid solution containing 20% methanol, followed by conversion from an acidic to alkaline solution, and then extraction using a diatomaceous earth column.
Results: We analyzed certain PAAs in 38 synthetic organic colorants, resulting in the detection of 2,4-dimethylaniline in four samples at 1.2 to 19 μg/g, o-toluidine in three samples at 1.0 to 3.4 μg/g, p-phenylazoaniline in two samples at 74 to 305 μg/g, and, in one sample each, 2,4,5-trimethylaniline (13 μg/g), 5-nitro-o-toluidine (12 μg/g), and 2-methyl-4–(2-tolylazo)aniline (13 μg/g). Nearly all PAAs were determined to be starting materials for colorant synthesis, although p-phenylazoaniline in Yellow No. 407 was apparently a byproduct formed during synthesis. For Red No. 225, in which high concentrations of p-phenylazoaniline were detected, additional samples were purchased from five companies, and p-phenylazoaniline was detected at concentrations of 88 to 370 μg/g in all samples.
Conclusions: A method to analyze certain PAAs contained as impurities in synthetic organic colorants was developed, and the actual status of them in colorants was clarified.
Highlights: The analytical method developed in this study for the determination of certain PAAs contained as impurities in synthetic organic colorants may be used to improve the safety of colorants.

Synthetic organic colorants are used worldwide to color foods, cosmetics, fibers, papers, leathers, and other products. Among these, azo colorants are widely preferred in many countries because of their low cost and rich variety of more than 3000 types. They represent 65% of the total world colorant market (1). Some azo dyes are known to undergo chemical or enzymatic reduction reactions on the skin surface, in bacteria in the intestines, or in the liver, resulting in the cleavage of azo groups to form primary aromatic amines (PAAs), which have been pointed out to be carcinogenic or potentially carcinogenic (2–4). These PAAs

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are generally referred to as specific PAAs, and the use of azo dyes that produce them in textiles and other products is regulated in various countries (5, 6). In Japan, the Act on Control of Household Products Containing Harmful Substances enacted in April 2015 has provided regulations on azo compounds containing azo colorants that may form certain PAAs, including a limit on their use in fibers and leathers (≤30 μg/g for individual certain PAAs) (7).

Concern for the effects of these compounds on human health has led to regulations on the use of azo colorants that contain certain PAAs in their structure and may form certain PAAs via reductive reactions. However, even in the case of organic synthetic colorants that do not contain certain PAAs in their chemical structure, the possibility of containing them as impurities during synthesis cannot be denied. Therefore, the aim of this study was to develop an analytical method for determining the certain PAAs contained as impurities in synthetic organic colorants and to clarify the actual status of them in colorants.

Experimental

Samples

In total, 38 synthetic organic colorants were analyzed. Some of these colorants either contain certain PAAs in their structures or these compounds are present among the starting materials for their synthesis. They were voluntarily provided to us by their producers or purchased as commercial reagents. Table 1 shows the name, color index, and chemial abstracts service registry number (CASRN) of each colorant. Red No. 225 samples sold as experimental-grade reagents were purchased separately from five different companies for analysis.

Reagents

A total of 24 certain PAAs were measured, as specified under the Japan Act on Control of Household Products Containing Harmful Substances. Table 2 shows the compound name, CAS number, and supplier of each PAA. Naphthalene-d_8, which is used as an internal standard substance, was obtained at environmental analytical grade from Kanto Chemical (Tokyo, Japan). Benzidine-d_8 was purchased from Cambridge Isotope Laboratories (Tewksbury, MA); methyl-tert-butyl ether (MTBE) and methanol for residual pesticide analysis were obtained from Kanto Chemical and Sigma-Aldrich Japan (Tokyo, Japan), respectively; anhydrous sodium sulfate was obtained from Sigma-Aldrich (Japan); hydrochloric acid and 4 mol/L aqueous sodium hydroxide solution for harmful metal measurements and volumetric analysis, respectively, were purchased from Fujifilm Wako Pure Chemical Ind. (Osaka, Japan); polyethylene glycol 300 (PEG300) was also obtained from Fujifilm Wako Pure Chemical Ind. (Osaka, Japan). The diatomaceous earth column was Chem Elut (volume = 20 mL) from Agilent Technologies (Santa Clara, CA). Ultrapure water was obtained using a Milli-Q® Advantage A10 water purification system (Merck Millipore, Tokyo, Japan).

Sample Processing

First, 50 mg of colorant sample was placed in a screw-cap glass container, and 10 mL of 1 mol/L hydrochloric acid containing 20% methanol was added. The screw cap was then sealed, and ultrasound extraction was conducted for 5 min. Then centrifugation was conducted at 1610 × *g* for 5 min, the liquid phase was filtered (Kiriyama Roshi No. 5C, Kiriyama Glass Co., Tokyo, Japan), and the filtrate was collected in an Erlenmeyer flask.

Next, MTBE liquid-liquid extraction (LLE) or diatomaceous earth column extraction (DAE) (8) was performed. For the LLE, 10 mL of 1 mol/L hydrochloric acid containing 20% methanol was added to the colorant after ultrasound extraction, and the sample was washed and filtered as described above. The resulting filtrates were combined, and after the addition of 5 mL of 5 mol/L aqueous sodium hydroxide followed by agitation, 10 mL of MTBE was added, followed by shaking for 15 min, and centrifugation was conducted for 5 min at 1610 × *g*. The supernatant was then collected. This LLE process conducted using MTBE was performed three times, and after dehydration using anhydrous sodium sulfate, the obtained MTBE phases were then combined, followed by concentration to approximately 1 mL in a warm bath at ≤40°C with a rotary evaporator. Then 50 μL of the MTBE solution containing the internal standard substances at 2 μg/mL was added, followed by measurement using a gas chromatograph-mass spectrometer (GC-MS).

For the DAE process, 6 mL of 1 mol/L hydrochloric acid containing 20% methanol was added to the colorant after ultrasound extraction, and the sample was washed and filtered as described above. The resulting filtrates were combined with the initial filtrate. Next, 4 mL of 5 mol/L aqueous sodium hydroxide was added to the resulting filtrate and agitated, followed by loading into the diatomaceous earth column and standing for 15 min. The Erlenmeyer flask that contained the filtrate was rinsed with 10 mL of MTBE, and this MTBE was also loaded into the diatomaceous earth column. Another 10 mL of MTBE was used to rinse the Erlenmeyer flask followed by the loading of this sample into the diatomaceous earth column, and, finally, 60 mL of MTBE was then loaded into the diatomaceous earth column. The MTBE solution eluted from the diatomaceous earth column was then concentrated to ≤5 mL in a warm bath at ≤40°C using a rotary evaporator. and the volume of the sample solution was adjusted to 5 mL with MTBE. Next, 50 μL of MTBE solution containing 1.0% PEG300 and 2 μg/mL internal standard substances was added to 1 mL of the obtained sample solution, followed by measurements using GC-MS.

GC-MS Conditions

The GC-MS analyses were performed using a Focus GC/DSQ II (Thermo Fisher Scientific, Waltham, MA) with DB-35MS (length: 30 m; I.D.: 0.25 mm; film thickness: 0.25 μm; Agilent Technologies) as the separation column. The samples (1 μL) were injected in splitless mode. The carrier gas was helium (flow rate: 1 mL/min), and the oven temperature was maintained at 55°C for 5 min, increased by 15°C/min to 230°C, 5°C/min to 290°C, and finally by 20°C/min to 310°C, and then the temperature was maintained for 5 min. The injector, transfer line, and ion source temperatures were 250, 280, and 250, respectively. The MS system was operated in the electron ionization (EI) mode at 70 eV, and analysis was carried out using the selected ion monitoring (SIM) mode. The retention times and quantifying and qualifying ions are listed in Table 3.

Results and Discussion

Synthetic organic colorants are categorized as hydrophobic dyes, hydrophilic dyes, or pigments based on their properties. For the hydrophilic dyes and pigments, we initially attempted extraction by adding the target PAAs and extracting using hexane (a nonpolar solvent), but this resulted in the insufficient

Table 1. List of investigated synthetic organic colorants

Name	CAS RN[a]	CI No.[b]	Synonym	Remark[c]
Black No. 401	1064-48-8	20470	Naphthol Blue Black	
Brown No. 201	1320-07-6	20170	Resorcin Brown	2,4-Xylidine was a synthesis starting material
Green No. 201	4403-90-1	61570	Alizarine Cyanine Green F	
Green No. 202	128-80-3	61565	Quinizarine Green SS	
Orange No. 204	3520-72-7	21110	Benzidine Orange G	3,3'-Dichlorobenzidine was a synthesis starting material
Orange No. 205	633-96-5	15510	Orange II	
Orange No. 402	523-44-4	14600	Orange I	
Orange No. 403	2646-17-5	12100	Orange SS	o-Toluidine was a synthesis starting material
Red No. 2	915-67-3	16185	Amaranth	
Red No. 102	2611-82-7	16255	New Coccine	
Red No. 201	5858-81-1	15850	Lithol Rubine B	
Red No. 202	5281-04-9	15850:1	Lithol Rubine BCA	
Red No. 203	2092-56-0	15585	Lake Red C	
Red No. 204	5160-02-1	15585:1	Lake Red CBA	
Red No. 205	1248-18-6	15630	Lithol Red	
Red No. 206	1103-39-5	15630:2	Lithol Red CA	
Red No. 207	1103-38-4	15630:1	Lithol Red BA	
Red No. 208	6371-67-1	15630:3	Lithol Red SR	
Red No. 220	6417-83-0	15880:1	Deep Maroon	
Red No. 221	2425-85-6	12120	Toluidine Red	
Red No. 225	85-86-9	26100	Sudan III	p-Phenylazoaniline was a synthesis starting material
Red No. 227	3567-66-6	17200	Fast Acid Magenta	
Red No. 401	6252-76-2	45190	Violamine R	o-Toluidine was a synthesis starting material
Red No. 404	6448-95-9	12315	Brilliant Fast Scarlet	5-Nitro-o-toluidine was a synthesis starting material
Red No. 405	7023-61-2	15865	Permanent Red F5R	
Red No. 501	85-83-6	26105	Scarlet Red NF	o-Toluidine was a synthesis starting material 2-Methyl-4-(2-tolylazo)aniline was a synthesis intermediate
Red No. 502	3564-09-8	16155	Ponceau 3R	2,4,5-Trimethylaniline was a synthesis starting material
Red No. 503	3761-53-3	16150	Ponceau R	2,4-Xylidine was a synthesis starting material
Red No. 504	4548-53-2	14700	Ponceau SX	
Red No. 505	3118-97-6	12140	Oil Red XO	2,4-Xylidine was a synthesis starting material
Violet No. 201	81-48-1	60725	Alizurine Purple SS	
Violet No. 401	4430-18-6	60730	Alizurol Purple	
Violet No. 403	6408-50-0	61520	Sudan Blue B	
Yellow No. 4	1934-21-0	19140	Tartrazine	
Yellow No. 5	2783-94-0	15985	Sunset Yellow FCF	
Yellow No. 205	6358-85-6	21090	Benzidine Yellow G	3,3'-Dichlorobenzidine was a synthesis starting material
Yellow No. 406	587-98-4	13065	Metanil Yellow	
Yellow No. 407	6359-82-6	18820	Fast Light Yellow 3G	

[a] Chemical abstract registry number.
[b] Color index number.
[c] Cited from Ref. (9).

recovery of several PAAs, thereby suggesting adsorption of PAAs. We therefore decided to investigate the use of hydrochloric acid for all extractions involving hydrophobic dyes, hydrophilic dyes, and pigments. By using only hydrochloric acid, however, a lack of affinity toward hydrophobic colorant surfaces may prevent efficient extraction. A small amount of alcohol was therefore added to improve the contact efficiency of the extractant to the colorant surface, and hydrochloric acid containing 20% methanol was used for extraction.

In the present study, two methods (LLE and DAE) for preparation of the sample solutions for GC-MS analysis after extraction using hydrochloric acid containing 20% methanol were investigated. The latter method is used for extraction from a phosphate buffer solution as a testing method for fiber products (ISO 14362-1 2017) (8). All target PAAs (40 μg/g) were added to four colorants (hydrophilic colorants: Orange No. 205 and 402; hydrophobic colorants: Red No. 205 and Green No. 202) that do not contain specific PAAs, and the recovery rates obtained using LLE and DAE were determined ($n = 3$). For Green No. 202, the recovery rate of o-toluidine could not be determined because p-toluidine is used as its synthesis starting material (9), which interfered in the o-toluidine analysis. Table 4 shows the recovery rate test results, as well as the required minimum recovery rate as specified by fiber product standards for several target PAAs when using a phosphate buffer solution (ISO 14362-1 2017) (8). By comparing the recovery rates obtained via LLE and DAE with these required values, several target PAAs analyzed by LLE failed to meet these requirements, whereas all requirements were satisfied by those investigated using DAE under all conditions. Moreover, for specific PAAs with no required minimum value, DAE clearly provided a better recovery rate than LLE. For undetermined reasons, 2,4-diaminoanisole in Green No. 202 showed a large coefficient of variation in the recovery rate obtained via DAE. In contrast, the several LLE recovery rates that were found to be 130% or higher may be attributed partly to response enhancement that accompanies the matrix effect of a

Table 2. List of investigated aromatic amines

No.	Name	CAS RN	IARC classification[a]	Supplier[b]
1	o-Toluidine	95-53-4	1	W
2	2,4-Dimethylaniline	95-68-1	3	T
3	2,6-Dimethylaniline	87-62-7	2B	T
4	o-Anisidine	90-04-0	2B	W
5	4-Chloroaniline	106-47-8	2B	A
6	2-Methoxy-5-methylaniline	120-71-8	2B	W
7	2,4,5-Trimethylaniline	137-17-7	3	A
8	4-Chloro-2-methylaniline	95-69-2	2A	S
9	2,4-Diaminotoluene	95-80-7	2B	T
10	2,4-Diaminoanisole	615-05-4	2B	W
11	2-Naphthylamine	91-59-8	1	S
12	2-Methyl-5-nitroaniline	99-55-8	3	S
13	4-Aminodiphenyl	92-67-1	1	S
14	p-Phenylazoaniline	60-09-3	2B	F
15	4,4'-Diaminodiphenylether	101-80-4	2B	S
16	4,4'-Methylenedianiline	101-77-9	2B	W
17	Benzidine	92-87-5	1	S
18	2-Methyl-4-(2-tolylazo)aniline	97-56-3	2B	S
19	4,4'-Diamino-3,3'-dimethyldiphenylmethane	838-88-0	2B	S
20	3,3'-Dimethylbenzidine	119-93-7	2B	A
21	4,4'-Diaminodiphenyl sulfide	139-65-1	2B	W
22	3,3'-Dichloro-4,4'-diaminodiphenylmethane	101-14-4	1	S
23	3,3'-Dichlorobenzidine	91-94-1	2B	A
24	3,3'-Dimethoxybenzidine	119-90-4	2B	W

[a] IARC classification groups: 1 = Carcinogenic to humans, 2A = Probably carcinogenic to humans, 2B = Possibly carcinogenic to humans, 3 = Not classifiable as carcinogenic to humans.
[b] W: Wako Pure Chemical Industries, Ltd., T: Tokyo Kasei Kogyo Co., Ltd., A: AccuStandard Inc., S: Sigma-Aldrich, F: Fulka.

Table 3. Retention times and quantifying and qualifying ions of target PAAs

Chemicals	Retention time, min	Quantifying ion, *m/z*	Qualifying ion, *m/z*
o-Toluidine	10.73	106	107
2,4-Dimethylaniline	11.78	121	120
2,6-Dimethylaniline	11.84	121	120
o-Anisidine	12.08	123	108
4-Chloroaniline	12.46	127	129
2-Methoxy-5-methylaniline	13.04	137	122
2,4,5-Trimethylaniline	13.09	120	135
4-Chloro-2-methylaniline	13.45	141	143
2,4-Diaminotoluene	14.64	121	122
2,4-Diaminoanisole	15.50	123	138
2-Naphthylamine	16.15	115	143
2-Methyl-5-nitroaniline	16.65	152	106
4-Aminodiphenyl	17.82	169	152
p-Phenylazoaniline	21.70	197	120
4,4'-Diaminodiphenylether	22.54	200	171
4,4'-Methylenedianiline	22.68	198	197
Benzidine	22.80	184	167
2-Methyl-4-(2-tolylazo)aniline	23.85	225	106
4,4'-Diamino-3,3'-dimethyldiphenylmethane	24.88	226	211
3,3'-Dimethylbenzidine	25.29	212	196
4,4'-Diaminodiphenyl sulfide	26.80	216	184
3,3'-Dichloro-4,4'-diaminodiphenylmethane	27.40	266	268
3,3'-Dichlorobenzidine	27.41	252	254
3,3'-Dimethoxybenzidine	27.60	244	201
Naphthalene-d_8[a]	11.94	136	
Benzidine-d_8[a]	22.75	192	

[a] Internal standard.

contaminant in the GC injection port. It is known that coexistence of PEG in a measurement solution can effectively suppress the occurrence of such matrix effects in the GC injection port (10). Accordingly, when the sample solution is adjusted to include 1.0% PEG300 at the time of GC injection in the DAE procedure, no anomalously high recovery rates comparable to those found using LLE occurred. Considering these findings, this DAE process was used to investigate certain PAAs in synthetic organic pigments. Total current ion chromatogram of the standard solution containing 1.0% PEG 300 is shown in Figure 1. Good linearity of standard curve of every chemical was observed in the concentration range of 0.01–1 μg/mL, and we took the value obtained by converting the calibration curve minimum (0.01 μg/mL) to the actual sample value (LLE: 0.2 μg/g; DAE: 1.0 μg/g) as the lower limit of quantification (LOQ).

We applied the developed analytical method to evaluate the PAAs in 38 synthetic organic colorants. We found 2,4-dimethylaniline in four samples at 1.2 to 19 μg/g, o-toluidine in three samples at 1.0–3.4 μg/g, p-phenylazoaniline in two samples at 74–305 μg/g, and, in one sample each, 2,4,5-trimethylaniline

Table 4. Liquid-liquid extraction (LLE) and diatomaceous earth column extraction (DAE) recovery rates

		Orange No. 205				Orange No. 402				Red No. 205				Green No. 202			
		LLE		DAE		LLE		DAE		LLE		DAE		LLE		DAE	
Chemicals	Recovery requirement[a]	Ave	CV	Ave	CV	Ave	CV	Ave	CV	Ave	CV	Ave	CV	Ave	CV	Ave	CV
o-Toluidine	50%	76	8.0	106	1.5	75	5.3	102	2.5	81	4.2	102	1.3	n.t.[b]	n.t.	n.t.	n.t.
2,4-Dimethylaniline		90	14	103	1.8	79	5.3	99	1.4	87	4.4	97	0.33	72	2.8	102	2.5
2,6-Dimethylaniline		73	8.6	98	1.0	70	5.7	96	2.5	65	3.9	90	1.1	69	2.0	98	2.5
o-Anisidine	70%	80	8.8	99	2.3	68	2.2	98	2.0	75	3.9	94	0.91	73	1.2	100	1.3
4-Chloroaniline	70%	78	9.4	99	2.0	77	3.7	98	2.3	77	3.6	95	0.34	89	2.0	101	1.1
2-Methoxy-5-methylaniline	70%	81	11	100	3.6	73	4.2	98	2.7	82	3.0	94	0.44	81	5.2	103	1.8
2,4,5-Trimethylaniline	70%	80	12	99	2.9	72	2.2	92	1.4	58	4.6	72	1.7	79	2.7	101	1.6
4-Chloro-2-methylaniline	70%	76	9.2	103	2.9	75	3.1	98	2.5	76	3.5	96	1.5	80	4.0	103	1.7
2,4-Diaminotoluene	50%	56	5.7	100	1.7	29	14	102	1.6	67	6.1	95	0.64	46	2.1	97	2.5
2,4-Diaminoanisole	20%	n.d.[c]	–[d]	73	7.9	n.d.	–	86	5.1	56	17	78	1.4	43	6.3	37	84
2-Naphthylamine	70%	67	2.2	107	2.3	86	5.5	87	1.3	108	2.0	83	0.46	92	3.8	106	0.41
2-Methyl-5-nitroaniline		85	2.9	111	0.60	97	3.7	106	1.9	108	1.3	101	1.2	96	2.5	114	1.4
4-Aminodiphenyl	70%	97	9.6	100	0.97	62	6.4	93	3.1	145	8.7	72	2.2	60	3.4	101	1.4
p-Phenylazoaniline		51	5.0	101	1.5	n.d.	–	49	8.1	22	9.1	11	11	n.d.	–	105	1.5
4,4'-Diaminodiphenylether	70%	100	3.7	100	0.59	77	3.2	90	1.5	76	3.1	90	0.95	91	1.9	97	0.46
4,4'-Methylenedianiline	70%	101	4.6	99	0.50	76	3.8	94	2.0	87	1.1	92	2.1	87	6.2	101	1.1
Benzidine	70%	76	3.6	103	0.70	60	3.6	89	0.61	52	3.5	67	1.9	73	3.3	102	1.3
2-Methyl-4-(2-tolylazo)aniline		58	5.4	109	0.14	50	7.5	57	7.4	20	11	7.8	11	58	9.8	106	1.8
4,4'-Diamino-3,3'-dimethyldiphenylmethane	70%	130	6.7	108	0.70	71	5.2	98	0.62	171	5.5	98	1.8	79	4.0	106	1.1
3,3'-Dimethylbenzidine	70%	90	3.1	104	0.14	68	5.2	86	0.20	101	6.1	66	2.1	81	3.0	104	0.30
4,4'-Diaminodiphenyl sulfide	70%	76	3.9	103	2.4	77	2.4	88	0.57	106	8.2	86	1.4	92	1.8	97	1.7
3,3'-Dichloro-4,4'-diaminodiphenylmethane	70%	85	3.0	101	1.3	58	7.9	100	3.0	72	8.2	92	2.6	59	5.4	105	0.81
3,3'-Dichlorobenzidine	70%	100	5.1	96	2.2	61	5.1	89	2.1	135	11	57	1.9	63	3.7	102	0.87
3,3'-Dimethoxybenzidine	70%	68	3.4	107	1.2	79	6.7	88	3.0	103	8.1	74	4.2	105	4.5	101	1.9

[a] Required by ISO 14362-1.[9]
[b] n.t. = Not tested.
[c] n.d. = Not detected.
[d] – = Not calculated.

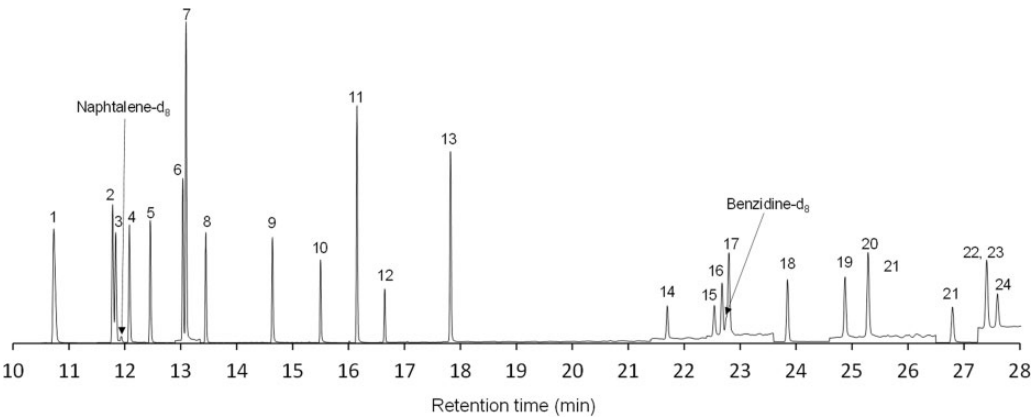

Figure 1. Total current ion chromatogram of standard solution containing 0.05% PEG300 (0.4 μg/mL, internal standard: 0.1 μg/mL). Peak numbers correspond to assignments in Table 2.

Table 5. Target PAA concentrations in synthetic organic colorants

	Concentrations of PAAs, μg/g					
	o-Toluidine	2,4-Dimethylaniline	p-Phenylazoaniline	2,4, 5-Trimethylaniline	2-Methyl-5-nitroaniline	2-Methyl-4–(2-tolylazo) aniline
Brown No. 201	–[a]	8.3	–	–	–	–
Red No. 225	–	–	305	–	–	–
Orange No. 403	2.7	–	–	–	–	–
Red No. 404	–	–	–	–	12	–
Red No. 501	3.4	–	–	–	–	13
Red No. 502	–	–	–	13	–	–
Red No. 503	1.0	19	–	–	–	–
Red No. 504	–	1.2	–	–	–	–
Red No. 505	–	3.6	–	–	–	–
Yellow No. 407	–	–	74	–	–	–

[a] Below LOQ.

(13 μg/g), 2-methyl-5-nitroaniline (12 μg/g), and 2-methyl-4–(2-tolylazo)aniline (13 μg/g) (Table 5). Among the detected PAAs, those other than 2-methyl-4–(2-tolylazo)aniline in Red No. 501, o-toluidine in Red No. 503, and p-phenylazoaniline in Yellow No. 407 were detected in the synthetic organic colorants for which they were used as starting materials during synthesis. Additionally, 2-methyl-4–(2-tolylazo)aniline in Red No. 501 forms as an intermediate in the synthesis process with o-toluidine as a starting material (9). These PAAs can therefore be considered residuals of substances that were unreacted during synthesis. In contrast, the o-toluidine present in Red No. 503 may have been present as an impurity in the 2,4-dimethylaniline starting material for synthesis, but o-toluidine was not detected in another colorant that similarly uses 2,4-dimethylaniline as a starting material; therefore, the origin of this compound is not clearly understood. The p-phenylazoaniline present in Yellow No. 407 is neither used as a synthesis starting material nor present as an intermediate. However, after diazotization, aniline used as starting substance undergoes a coupling reaction with 3-methyl-1-(p-sulfophenyl)-5-pyrazolone (9), in which inter-aniline coupling may have occurred, thereby forming p-phenylazoaniline.

The p-phenylazoaniline present in Red No. 225 showed the highest concentration of all detected specific PAAs. Additional Red No. 225 samples, which are marketed as experiment-grade reagents, were purchased from five different companies, and their assessment all resulted in the detection of p-phenylazoaniline at concentrations of 88 to 370 μg/g. Therefore, in synthetic organic colorants Red No. 225, p-phenylazoaniline is present as impurities in concentrations that can be expected to vary among different producers and distributors. The synthetic organic colorants assessed in the present study are used in cosmetics and other products. However, because low colorant contents are present in these products, the specific PAAs quantified in the present study cannot be immediately inferred to be harmful to the health of humans. The concentration of specific PAAs as impurities may vary depending on the producer and distributor, and the resulting products may be orally ingested or subjected to prolonged skin contact, so these PAAs as impurities should be regulated. The method developed in the present study for the examination of specific PAAs in synthetic organic colorants can therefore contribute to improving the safety of colorants.

Conclusions

An analytical method was developed and applied in this study to analyze the presence of specific PAAs as impurities in synthetic organic colorants. This method involves the ultrasound extraction of the synthetic organic colorant using hydrochloric acid containing 20% methanol, followed by conversion from acidic to alkaline conditions and subsequent extraction using a diatomaceous earth column. This experimental method was applied to assess specific PAAs in 38 synthetic organic colorants, resulting in the detection of 2,4-dimethylaniline in four samples at 1.2–19 μg/g, o-toluidine in three samples at 1.0–3.4 μg/g, p-phenylazoaniline in two samples at 74–305 μg/g, and, in one sample each, 2,4,5-trimethylaniline (13 μg/g), 5-nitro-o-toluidine (12 μg/g), and 2-methyl-4–(2-tolylazo)aniline (13 μg/g). Although nearly all PAAs were starting materials for colorant synthesis, p-phenylazoaniline in Yellow No. 407 was estimated to be a byproduct that was formed during synthesis. For Red No. 225, in which high concentrations of p-phenylazoaniline were detected, additional samples were purchased from five different companies. The analyses of these samples all exhibited the detection of p-phenylazoaniline at concentrations of 88–370 μg/g. The method developed in the present study for the assessment of specific PAAs present in synthetic organic colorants may contribute to improving the safety of colorants.

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Conflict of Interest

All authors declare no conflict of interest.

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