REVIEW　　　　　　　　　　　　　　　　　　　　　　　　　　**Open Access**

Oxidative transformation processes and products of *para*-phenylenediamine (PPD) and *para*-toluenediamine (PTD)—a review

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Abstract

para-Phenylendiamine (PPD) and its ring-methylated analogue, *para*-toluenediamine (PTD), are primary intermediates (precursors) for azo dye synthesis. Particularly, they are used in oxidative hair dye formulations and in henna tattoo inks. In the hair coloration process, the final permanent color develops during complex chemical reactions in the presence of an oxidant, e.g., hydrogen peroxide, and a coupler, e.g., resorcinol (benzene-1,3-diol). PPD is well known as the most important allergen associated with hair dye-related allergic contact dermatitis. PPD acts as a pre- or pro-hapten. It is (aut)oxidatively transformed into haptens, e.g., benzoquinone diimine, benzoquinone, or Bandrowski's Base. In the presence of adequate amounts of couplers, some haptens, e.g., Bandrowski's Base, are not formed, and others, e.g., benzoquinone diimine, are converted into non-sensitizing products, e.g., the desired dye. Under toxicological aspects, the illegal use of henna tattoo inks containing aryldiamines but no couplers is of high concern. Nevertheless, even during hair coloration at comparably high coupler concentration, the aryldiamines are not completely scavenged and transformed by the couplers. To understand and to assess the reactive and toxicological potential of PPD and PTD, a sound knowledge of their (aut)oxidation reactions and of the nature of formed intermediates and (by)products is crucial. Thus, this review pursues the aim to provide a synopsis of the current state of knowledge of (aut)oxidative reactions of PPD and PTD in the absence of a coupler. Monomeric and oligomeric reaction products of each compound are presented together with the reaction conditions and analytical techniques applied for their generation and identification. Analytical data of these transformation products, e.g., electrochemical potentials, UV/VIS absorbance maxima, and mass spectrometric data, are tabulated. The certainty of the substance identification is rated with respect to the performance of the applied analytical techniques, to the concordance of results achieved by different methods, and to the traceability of the analytical procedure. Reaction pathways are outlined for both compounds. An integrated reaction scheme is drawn for PPD. Finally, intrinsic difficulties of process and product elucidation are discussed, and some conclusions are drawn pointing to several knowledge gaps and to new analytical approaches, potentially helpful to fill them.

Keywords: Aryldiamines; Diaminobenzenes; *para*-Phenylenediamine (PPD); *para*-Toluenediamine (PTD); Azo dyes; Hair dyes; Henna tattoo inks; Oxidative transformation products; Bandrowski's Base; Benzoquinone diimine

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Review

Introduction

With the first application of *para*-phenylenediamine (PPD) for the generation of an azo dye in the year 1863, a new area in the synthesis of organic dyes started up [1]. Nowadays PPD is still in use as intermediate for the production of technical azo dyes and printing inks [2, 3]. It serves as developer in oxidative hair dye formulations and acts as a color-deepening component of henna tattoos. PPD has found further applications in the synthesis of pharmaceutics, antioxidants, and vulcanization accelerators as well as in the rubber and plastics industry [4, 5]. The PPD methyl analogue, *para*-toluenediamine (PTD), firstly synthesized in 1877, has a similar application profile but is produced in lesser amounts, e.g., as intermediate for the synthesis of the azo dyes Basic Red 2 and Acid Brown 103 [6]. In the last years, PTD has widely replaced PPD as a hair dye component, at least in Europe [7].

As aryldiamines, PPD, PTD, and their transformation products are of toxicological and environmental concern. They are assessed as toxic [8], mutagenic in vitro [9], and carcinogenic [9–11]. The International Agency for Research on Cancer (IARC) has categorized various phenylenediamines as human carcinogens [12]. In the year 2012, the "Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area" of the German Research Foundation (DFG) has assessed PPD as a carcinogenic occupational substance (category 3B) [13]. A specific feature of phenylenediamines, especially of PPD, is their allergic potential [14, 15]. PPD is known as the most important allergen associated with hair dye-related allergic contact dermatitis [16, 17]. It is a pre- or pro-hapten and converted into several haptens by various (aut)oxidative reactions. With respect to occupational health and consumer health requirements, the usage of PPD and PTD in hair and tattoo dye formulations is subject to various regulations. The European Economic Community Cosmetics Directive has set limit values for the maximal concentration of phenylenediamines in hair dyes to 6 % (PPD) and 10 % (PTD), provided that the active dye formulation contains an excess of coupling compounds [18]. The toxicological potential of technical and commercial formulations of aryldiamines is not only coined by the pure compounds themselves but also by side products of their technical synthesis and by chemical (aut)oxidation products formed during post production handling, product formulation, storage, application, or environmental exposure [19]. In contact with air or oxidizing agents like peroxides, phenylenediamines are easily transformed into complex mixtures of monomeric, dimeric, oligomeric, and polymeric oxidation products. Furthermore, the amines might undergo photolytic or photooxidative degradation when exposed to sunlight [20, 21]. In properly composed oxidative hair dye formulations, the aryldiamines are efficiently converted into the desired permanent dye by reaction with the coupler component avoiding the formation of toxic byproducts, e.g., Bandrowski's Base [2, 22]. Nevertheless, even under those conditions, the aryldiamines are not completely scavenged and consumed by the couplers so that a certain reactivity potential might persist. Consequently, henna tattoo coloration formulations containing aryldiamines, e.g., PPD, without a coupler, are of high toxicological concern, since under those conditions, potentially mutagenic and carcinogenic compounds are formed by autoxidation processes of the diaminobenzenes. The current status of the PPD risk assessment is briefly summarized in [23]. Thus, a final evaluation of the toxic properties and environmental behavior of phenylenediamines requires an in-depth knowledge of their transformation products formed under the respective conditions.
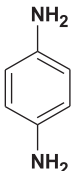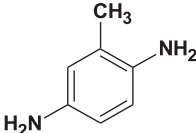
The elucidation of their reaction pathways and products has a long history, starting with the pioneering work of Bandrowski [24]. The topic remains the object of ongoing research [25, 26].

This review aims at a comprehensive synopsis of (tentatively) identified oxidation products of PPD and PTD with respect to the specific reaction conditions, which led to their generation and identification. The focus lies on abiotic processes and transformation products assumed to be relevant for human exposure to aryldiamines. Neither a detailed toxicological assessment of their oxidation products nor the characterization of specific physiological reaction conditions, e.g., on the surfaces of hair and skin, is within the scope of this review. As far as investigated, the reaction products and intermediates are integrated in reaction schemes, combining different reaction pathways. The reliability of the analytical determination of the transformation products is assessed with respect to the used instrumentation, the traceability of the examination conditions, and the concordance of independently attained data. Core data of UV–VIS and MS spectra of transformation products are collected and tabulated. Finally, the review article intends to point out further research needs, e.g., in cases of insufficiently characterized reaction products or of incomplete reaction pathways.

Substance properties

The most important substance properties of PPD and PTD are summarized in Table 1. In hair dyes, the application of their respective hydrochloric acid or sulfate salts like PPD × 2 HCl or PTD sulfate is common also. Due to the small molecular surface and the amino groups, the water solubility is high and pH-dependent. Vice versa, the K_{ow} values are low. The documented pK_a values of PPD vary according to the consulted reference. For instance, the reported pK_{a1} value of PPD stretches from 2.8 to 3.29 and the pK_{a2} from 6.08 to 6.44 [27–29].

Table 1 Properties of PPD and PTD

	para-Phenylenediamine (**PPD**)	*para*-Toluenediamine (**PTD**)
Names and synonyms	1,4-Benzenediamine	2-Methyl-para-phenylenediamine
	1,4-Diaminobenzene	2,5-Diaminotoluene
	4-Aminoaniline	2-Methyl-1,4-diaminobenzene
Structural formula		
CAS number	106-50-3	95-75-5
Molecular mass ($g \cdot mol^{-1}$)	108.14	122.19
Aggregate state (STP)	Solid (colorless or light red crystals)	Solid (light yellow crystals)
Melting point (°C)	139.7	64
Boiling point (°C)	267	273.7
Hazard symbols		
Water solubility at 25 °C ($g \, L^{-1}$)	37.0	72.4
log K_{OW}	−0.36	0.16
LD_{50} (oral, rat)	80 $mg \cdot kg^{-1}$	102 $mg \cdot kg^{-1}$
LD_{50} (inhalation, rat)	0.92 mg/l/4 h	
pK_{a1}	2.8 [27], 3.29 [28]	2.77
pK_{a2}	6.08 [28], 6.20 [27], 6.44 [29]	6.39

Data from SCPP [30, 31] and ChemSpider, if not stated otherwise

In studies of the European Scientific Committee on Consumer Products (SCCP), the purity of commercial batches of PPD and PTD used in hair dye formulations was examined and found to exceed 98 % [30, 31]. According to these reports, PPD was contaminated by o- and m-PPD (<200 $mg \, kg^{-1}$), o-aminophenole (<500 $mg \, kg^{-1}$), and aniline (<50 $mg \, kg^{-1}$). Turesky et al. determined up to 0.5 $mg \, kg^{-1}$ 4-aminobiphenyl both in commercially available products containing PPD and in the pure chemical [32]. Up to 50 $mg \, kg^{-1}$ o-toluidine was detected in PTD batches.

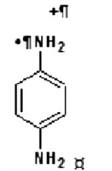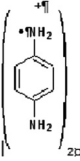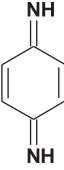### Formation and analytical characterization of PPD and PTD oxidation products

Originally, the analytical characterization of oxidation products of phenylenediamines intended to elucidate reaction pathways leading to the formation of azo dyes. Further research aspects were the minimization of the formation of byproducts and the optimization of the reactions yields, required for an improvement of the industrial azo dye synthesis. Later on, the focus widened to cover toxicological aspects, initially concentrating on the generation of Bandrowski's Base (BB), a very strong sensitizing agent. Other research aspects also shaped by toxicological concerns are the formation of adducts between aryl(di)amines and amino acids or peptides.
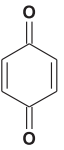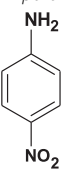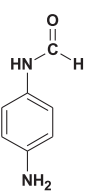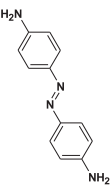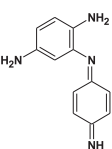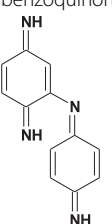
A visual sign of the reactivity of phenylenediamines is the development or change of color of their aqueous, oxygen containing, or air-exposed solutions within a few hours. Within a few hours, the solutions turn dark brown and, subsequently, a black-brown precipitate forms. The formation of the reaction products and thus the transformation of PPD and PTD are pH-dependent and accelerated under alkaline conditions. Stored at 4 °C, aqueous PPD solutions remain stable for more than 24 h [33].

So far as retrievable, oxidation products of PPD and PTD documented in literature are listed in Tables 2 and 3, respectively. The most important facts of their formation conditions, e.g., the type of the reacting oxidant, are documented as well. In addition to analytical details, the certainty and reliability of the analytical findings are rated by a five-level scale, ranging from highly reliable (++) to insufficiently reliable (−−). For details, see footnote of Table 2. It is not surprising to observe that a certain negative correlation between the reliability of structure elucidation and the age of the report exists. Clearly, the main reason is the missing availability of high performance separation and detection techniques in former times, but other reasons, e.g.,
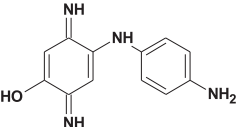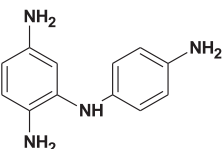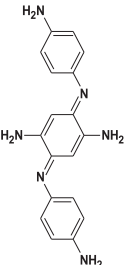
Table 2 Oxidation products of PPD

Name, molecular mass (amu), and structural formula	Reaction conditions	Analytical method	Relative analytical reliability[a]	References
Monomeric compounds				
Semibenzoquinone diimine radical cation (SBQDIRC) 108.14	ox. ag.: ferricyanide, pH 8 (phosphate buffer)	UV–VIS spectroscopy, EPR spectroscopy	+	[35]
	pH 4.8 (acetate buffer)	CVA, EPR spectroscopy	+	[34]
	pH 2–8 (Britton-Robinson buffer)	Electrolysis at GCE- or Pt-electrode, EPR spectroscopy	+	[38]
	solv.: CH_2Cl_2 and ACN	Electrochemical oxidation at GCE, UV–VIS spectra	−	[37]
	pH 7.0 (phosphate buffer)	Oxidation at a GCE modified with MnO_2, UV–VIS spectroscopy, 415–425 nm	−	[36]
	pH 7.0 (water), 30 min reaction time, drying until solid formation under vacuum	Adsorption on Au- and Cu-surfaces of nanoparticles, UV–VIS spectroscopy, surface enhanced Raman spectroscopy (SERS)	+	[46]
	solv.: ACN	Oxidation at Pt electrode, EPR spectroscopy	+	[39]
	ox. ag.: OH• and N_3•, pH 8 (buffer)	UV–VIS spectroscopy, 300, 370, 460, and 490 nm, Raman spectroscopy, 1430 and 1650 nm	+	[45]
	solv.: ACN	CVA, Pt electrode, EPR spectroscopy	+	[40]
	ox. ag.: Br_2 or photolysis, solv.: methanol, ethanol, pH 7 (water)	UV–VIS, IR, and Raman spectroscopy, 300, 460, 490, and 560 nm	o	[43]
	ox. ag.: Br_2 pH 4.6 (20 % acetate buffer + 80 % methanol)	UV–VIS spectroscopy, 462 and 479 nm	−−	[41]
	Photolysis, ox. ag.: Br_2, solv.: ethanol, −72 °C, −130 °C, −196 °C	UV–VIS spectroscopy, 465 and 500 nm	−−	[71]
	ox. ag.: Br_2 solv.: ethanol	UV–VIS spectroscopy, 463 and 490 nm, resonance Raman and Raman spectroscopy	o	[42]
Dimeric SBQDIRC 216.29	ox. ag.: Br_2, UV photolysis, pH 7 (water), ethanol (−72 °C); methanol	UV–VIS spectroscopy, 600 nm, Raman spectroscopy	−−	[43]
	Photolysis, solv.: ethanol, −72 °C, −130 °C, −196 °C	UV–VIS spectroscopy, 600 nm	−−	[71]
	solv.: ethanol −72 °C, 25 °C	Equilibrium, SBQDIRC and (SBQDIRC)$_2$, UV–VIS and resonance Raman spectroscopy, 448 nm (monomer), 600 nm, 670 nm (dimer)	−	[44]
para-Benzoquinone diimine (BQDI) 106.13	ox. ag.: ferricyanide, pH 8 (buffer)	UV–VIS spectroscopy 257 and 265.5 nm	−−	[35]
	ox. ag.: O_2, ferricyanide, pH 7 (water), 30 min	HPLC-MS/MS, m/z: 107 amu $[M + H]^+$ fragments: 80 and 53 amu	+	[49]
	ox. ag.: MnO_2, pH 7 (phosphate buffer)	CVA, oxidation at an electrode modified with MnO_2, UV–VIS spectroscopy, 276, 319, and 465 nm	−−	[36]

Table 2 Oxidation products of PPD *(Continued)*

	pH 7.4 (30 % ACN/70 % ammonium acetate)	Electrochemical oxidation, EC-ESI-MS-TOF, m/z: 107 amu [M + H]+	++	[25]
para-Benzoquinone (PBQ) 108.10	ox. ag.: MnO₂, solv.: benzene	UV and IR spectroscopy, melting point	○	[73]
para-Nitroaniline138.13	ox. ag.: H₂O₂, ammoniacal solution, filtration of precipitates, dissolution in DMF, TLC separation	IR spectroscopy, melting point	○	[55]
4-Aminophenyl formamide 136.15	Culture medium	HPLC-MS; ¹³C-, UV-, MS-detection, m/z: 137 amu [M + H]+	−	[8]
	Culture medium	HPLC-MS/MS, d⁴-PPD, m/z: 137/141 amu [M + H]+	○	[56]

Dimeric compounds

4,4′-Diamino-azobenzene (DAB) 212.25	ox. ag.: MnO₂, solv.: benzene, 30 % yield	IR and UV spectroscopy of the isolated substance	○	[73]
	ox. ag.: H₂O₂, ammoniacal solution, filtration of precipitates, dissolution in DMF, TLC separation	Rf-value (TLC), UV- and IR spectroscopy, elemental analysis, melting point	+	[55]
	ox. ag.: O₂, ferricyanide, aqueous solution, 30 min	HPLC-MS/MS m/z: 198 amu [M + H]+	○	[49]
	pH 7.4 (30 % ACN/70 % ammonium acetate)	Electrochemical oxidation, EC-ESI-MS-TOF, m/z: 213 amu [M + H]+	++	[25]
2-(1′,4′-Benzoquinone diimine)-4-aminoaniline 212.25	pH 7.4 (30 % ACN/70 % NH₄Ac)	Electrochemical oxidation, EC-ESI-MS-TOF, m/z: 213 amu [M + H]+	○	[25]
2-(1,4-Benzoquinone diimine)-1,4-benzoquinone diimine 210,24	pH 7.4 (30 % ACN/70 % NH₄Ac)	Electrochemical oxidation, EC-ESI-MS-TOF, m/z: 211 amu [M + H]+	○	[25]

Table 2 Oxidation products of PPD *(Continued)*

2-(4'-Aminoaniline)-5-hydroxy-1,4-benzoquinone diimine 228.25	ox. ag.: H_2O_2, ammoniacal solution, filtration of precipitates, dissolution in DMF, TLC separation	UV, NMR, and IR spectroscopy, 460 nm, 550 nm, melting point, elemental analysis	+	[55]
2-(4'-Aminoaniline)-1,4-diaminobenzene 214.27	solv.: ACN	Electrochemical oxidation, coulometry at Pt electrode	––	[58]
Trimeric product				
Bandrowski's Base *N',N'*-bis-(4-Aminophenyl)-2,5-diamino-1,4-benzoquinone diimine 318.38	ox. ag.: O_2, ferricyanide	Elemental analysis	––	[24]
	Culture medium	PPD transformation by dendritic cells, reaction time 16 h, HPLC-MS, HPLC radio detection, HPLC-UV (254 nm)	+	[2]
	ox. ag.: H_2O_2 ammoniacal solution, filtration of precipitates, dissolution in DMF, TLC separation	NMR, IR, and UV–VIS spectroscopy (~460 nm)	+	[55]
	ox. ag.: H_2O_2 pH 9.5 (sodium carbonate); filtration of precipitates	Elemental analysis, TLC, IR, and UV–VIS spectroscopy	○	[59]
	pH 7.4 (30 % ACN/70 % NH_4Ac)	Electrochemical oxidation, EC-ESI-MS-TOF, m/z: 319 amu $[M + H]^+$	○	[25]
	ox. ag.: O_2, ferricyanide, pH 7 (water), 30-min reaction time, pH 4 (buffer), 1-h reaction time	HPLC-MS/MS, m/z: 319 amu $[M + H]^+$, confirmation of substance identity by comparison with standard material	++	[49]
	pH-dependency (pH 4, 5, 6, 7, and 9) of BB formation	HPLC-DAD (480 nm)	+	[49]
	ox. ag.: H_2O_2 pH 2.2 (HCl), pH 9.9 (ammoniacal solution)	Ames Test, TLC separation, UV–VIS spectroscopy, comparison with standard	○	[63]
	ox. ag.: photolysis, solv.: DMSO, reaction time 1–4 h	Ames Test, TLC separation, detection with UV and IR spectroscopy	–	[64]

ox. ag. oxidizing agent, *solv.* solvent, *ACN* acetonitrile, *CVA* cyclic voltammetry, *DAD* diode array detection, *DMF* dimethyl formamide, *DMSO* dimethyl sulfoxide, *EPR* electron paramagnetic resonance spectroscopy, *ESI* electrospray ionization (MS interface), *GCE* glassy carbon electrode, *TOF* time-of-flight (MS technique)
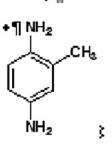[a]Five degrees of relative analytical reliability: ++: two independent high resolution (HR) spectroscopic methods, e.g., MS, NMR, EPR, or combination of one HR method with various others; +: one HR method; ○: one HR method but with interferences; combination of methods with lower resolution or lesser specificity; –: one method with lower resolution/lesser specificity; ––: analysis of reaction mixtures with non-specific or low performance methods
The experimental design, number of different reaction conditions, and number of replicates or reproducibility tests are also taken into account

focus on specific research interests, have to be considered also. Thus, it is one of the issues of this review to provide an impetus for a re-evaluation of older analytical results. In this context, it should be mentioned that the number of studies related to the transformation of PPD surpasses those dealing with PTD by far.

Tables 4, 5, and 6 contain analytical data characterizing oxidation products of PPD and PTD. Some studies dealt with the electrochemical behavior of PPD. Medium-dependent, e.g., pH value, solvent type, and type and concentration of added electrolytes, electrochemical potentials of the aryldiamines and of their transformation

Table 3 Oxidation products of PTD

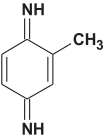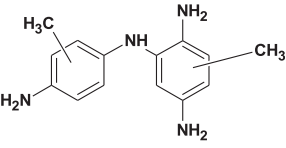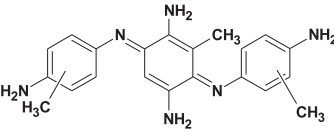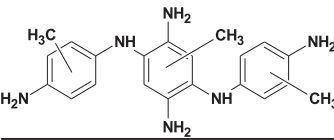| Name, molecular mass (amu), and structural formula	Reaction conditions	Analytical method	Relative analytical reliability[a]	References
Semitoluquinone diimine radical cation (STQDIRC) 122.17	Buffer systems[b] pH 2, 4, 7, and 10	CVA, Pt electrode, UV–VIS and EPR spectroscopy	+	[47]
	ox. ag.: Br_2 acetate buffer pH 4.6	UV–VIS spectroscopy, 466 nm	–	[41]
Toluquinone diimine (TQDI) 120.15	Buffer systems[b] pH 2, 4, 7, and 10	CVA, Pt electrode, UV–VIS spectroscopy	–	[47]
2-(4′-Amino-x′-methylphenyl)amino-x′-methyl-PPD 242.32	Buffer systems[b] pH 2, 4, 7, and 10	CVA, Pt electrode, UV–VIS and EPR spectroscopy	+	[47]
PTD-Bandrowski's Base analogue 360.46	Buffer systems[b] pH 2, 4, 7, and 10	CVA, Pt electrode, UV–VIS spectroscopy	–	[47]
Triphenylamine derivative 362.48	Buffer systems[b] pH 2, 4, 7, and 10	CVA, Pt electrode, UV–VIS spectroscopy	–	[47]

[a]Five degrees of relative analytical reliability: ++: two independent high resolution (HR) spectroscopic methods, e.g., MS, NMR, EPR, or combination of one HR method with various others; +: one HR method; ○: one HR method but with interferences; combination of methods with lower resolution or lesser specificity; –: one method with lower resolution/lesser specificity; —: analysis of reaction mixtures with non-specific or low performance methods
[b]Buffer systems: pH 2: hydrogensulfate/sulfate buffer, pH 5: acetate buffer, pH 7: ammonia buffer, pH 10: hydrogencarbonate/carbonate buffer

products were measured. These data were used to purposefully generate the corresponding oxidation products electrochemically which were often analyzed by spectroscopic methods during their nascency. Table 4 lists the electrochemical potentials together with the type of the applied working electrode and the reaction medium. Table 5 presents UV–VIS absorbance data of PPD and of its oxidation products. Many of the newer studies made use of various mass spectrometric techniques often hyphenated to liquid chromatography. The essential results are summarized in Table 6.

Hereafter, the various oxidation products of PPD and PTD are presented together with the analytical investigations revealing their molecular structures.

Monomeric reaction products
Semibenzoquinone diimine radical cation
The unstable, very reactive semibenzoquinone diimine radical cation (SBQDIRC), which exists in equilibrium with its non-protonated, neutral form SBQDIR (Table 2), is the intermediate, which has been examined most intensively. It initiates the dye formation process, e.g., during hair coloration, by binding to a coupler molecule. It is supposed that the formation of the SBQDIRC is visually recognizable by a slightly yellow coloring of initially colorless aqueous PPD solutions. Piette et al. conducted one of the first studies applying electron paramagnetic resonance spectroscopy (EPR) to characterize PPD oxidation products formed in acetonitrile during redox potential shift experiments with cyclic

Table 4 Electrochemical PPD oxidation: conditions and potentials

Oxidation and redox potentials (V)	Reaction conditions	Reference
+0.59/+0.06	CVA, GCE-electrode, CH_2Cl_2	[37]
+1.13/+0.60		
+0.37/+0.29	CVA, GCE electrode, ACN	[37]
+0.89/+0.69		
pH 2: +0.43	Anodic VA, GCE electrode, pH 2–8: Britton-Robinson buffer	[38]
pH 5: +0.27		
pH 8: +0.02		
+0.45/+0.37	CVA, Pt electrode, 0.01 M $HClO_4$	[34]
+0.18		
+0.15/+0.20	CVA, GCE electrode, pH 7 (0.1 M phosphate buffer)	[36]
+0.10/+0.20	CVA, GC electrode modified with chitosan and MnO_2, pH 7 (0.1 M phosphate buffer)	
+0.55	anodic VA, Pt electrode, ACN	[39]
−0.10/−0.08	CVA, Pt electrode, ACN	[40]
+0.38/+0.42		
+0.28	CVA, Au electrode, ACN	[77]
+0.83		

ACN acetonitrile, *CVA* cyclic voltammetry, *GCE* glassy carbon electrode

Table 5 UV/VIS absorbance maxima of various PPD oxidation products

Analyte	λ_{max} (nm)	pH value, solvent	Reference
PPD	242, 307	8	[35]
	200, 251, 320	CH_3CN, CH_2Cl_2	[37]
p-Benzoquinone diimine (BQDI)	257	8	[35]
	255, 256	ether	[59]
	460	8	[35]
	247	7	[36]
	240, 303	8	[36]
	450	7	[46]
p-Benzoquinone monoamine (BQMI)	253	8	[35]
Semibenzoquinone diimine radical cation (SBQDIRC)	319, 420 (720)	CH_3CN, CH_2Cl_2	[37]
	462, 469	20 % acetate buffer pH 4.6/80 % methanol	[41]
	247, 350, 465	8	[36]
	512	7	[46]
	300, 460, 490, 560	7	[43]
Bandrowski's Base (BB)	460	9.2	[29]
	530–540	5.0	[29]
	400	Citrate buffer pH 2.3	[59]

voltammetry [34]. Later on, Corbett oxidized PPD in aqueous solution with ferricyanide [35]. The EPR spectra recorded during these experiments match those of the former very well. The UV absorbance of the aqueous solution was measured during the various stages of the redox cycle additionally (Table 5). Various other electrochemical investigations, performed in organic solvents and buffer mixtures, partially accompanied by EPR- and UV/VIS absorbance measurements, indicated the formation of a SBQDIR cation too but did not provide a higher reliability of their findings [36–40]. Melchior and Maki compared the EPR data of the SBQDIRC synthesized from [1]H-PPD and from ring deuterated PPD (d[4]-PPD), resulting in a structure confirmation due to almost identical hyperfine structure data [39]. Nonetheless, the spectroscopic analysis was always conducted not with the isolated intermediate but in the presence of unreacted PPD and of further oxidation products, which gives rise to spectral interferences. The same complication has to be considered evaluating the UV/VIS spectroscopic determination of products formed during the reaction of PPD with bromine in methanol and in acetate buffer [41]. Mayer et al. used the same oxidation reaction to generate the SBQDIRC for analysis with Raman spectroscopy [42]. Their data confirmed the presence of an imino group (−C = NH) and of a free electron. Ernstbrunner et al. used IR- and Raman spectroscopy to characterize PPD and d[4]-PPD photooxidation products

formed at −196 °C in an ethanolic glassy matrix during UV irradiation [43]. The interpretation of the spectroscopic data led to a confirmation of the SBQDIR cation structure. Their comparison of measured with modeled spectra resulted in a good match. They found hints for a $(PPD^+)_2$ dimer formation in aqueous solution at room temperature. Those hints were formerly noticed by Yokoyama and Maeda who reported an equilibrium between the monomeric and dimeric species in ethanol at − 72 °C, ascertained with Raman spectroscopy [44].

Sun et al. made another approach to proof the formation of SBQDIRC. They oxidized PPD with azide and hydroxyl radicals accompanied by UV–VIS and Raman spectroscopy [45]. Recently, de Carvalho et al. suspended Cu nanoparticles covered with a thin Cu oxide shell in aqueous PPD solutions [46]. The oxidative adsorption of PPD onto these particles led to the formation of surface bound SBQDIRC characterized in situ by surface enhanced Raman scattering (SERS) at an excitation wavelength of 1064 nm.

The diimine radical cation of PPD is not as stable as the Wurster's radical (radical cation of *N,N'*-substituted aryldiamines) due to the lack of stabilizing alkyl or aryl substituents at the nitrogen atoms. The analogous PPD radical does not form at strongly acidic pH (pH <2.0) [34]. It reaches highest stability at pH values between 3.5

Table 6 FIA-MS and HPLC-MS characterization of PPD oxidation products

Analyte	FIA medium or chromatographic conditions (separation column, eluent composition)	Monoisotopic mass $[M + H]^+$ (amu)	Fragment (amu)	Analytical method	Reference
p-Benzoquinone diimine (BQDI)	Waters Atlantis ACN/ammonium formate pH 7.5	107.06	80 $[M-HNC + H]^+$ 53 $[M-2HNC + H]^+ \triangleq [C_4H_4 + H^+]$	HPLC-MS/MS	[49]
	Fisher Scientific PepMAP Acclaim C_{18} (75 μm × 15 cm) ACN/0.05 % TFA	107.06		HPLC-MS/MS	[3]
	FIA/NH$_4$Ac (30:70)	107.06		EC-ESI-TOF-MS	[25]
SBQDIRC	FIA ACN/NH$_4$Ac (30:70)	108.07 $[M]^+$		EC-ESI-TOF-MS	[25]
PPD	Agilent Zorbax SB-C_{18} (4.6 × 250 mm) methanol/NH$_4$Ac pH 6.9	109.07		HPLC-MS/MS	[3]
	Agilent Zorbax SB-C_{18} (4.6 × 200 mm) methanol/NH$_4$Ac pH 6.9	109.07		HPLC-MS	[2]
	FIA ACN/NH$_4$Ac (30:70)	109.07		EC-ESI-TOF-MS	[25]
	direct injection	109.07	92.12 $[M-NH_3 + H]^+$	MALDI-MS-MS	[78]
Unknown	Waters Xterra MS C_{18} (4.6 × 150 mm, 5 μm) methanol/NH$_4$Ac, pH 7.2	137		HPLC-ESI-MS/MS	[8]
	Agilent Zorbax SB-C_{18} (4.6 × 200 mm) methanol/NH$_4$Ac pH 6.9	137		HPLC-MS	[2]
4-Aminophenyl formamide	Waters X-Bridge Phenyl (2.1 × 150 mm, 3.5 μm) methanol/NH$_4$Ac, pH 8	137.07		HPLC-ESI-MS	[56]
Dimeric oxidation products	Waters Atlantis ACN/ammonium formate pH 7.5	198		HPLC-MS/MS	[49]
2-(1,4-Benzoquinone diimine)-1,4-benzoquinone diimine	FIA ACN/NH$_4$Ac (30:70)	211.09		EC-ESI-TOF-MS	[25]
2-(1',4'-Benzoquinone diimine)-4-aminoaniline	FIA ACN/NH$_4$Ac (30:70)	213.11	198.10 $[M-NH + H]^+$ 183.09 $[M-2NH + H]^+$	EC-ESI-TOF-MS	[25]
Bandrowski's Base	Waters Atlantis ACN/ammonium formate pH 7.5	319.17		HPLC-MS/MS	[49]
	Zorbax SB-C_{18} (4.6 × 200 mm) methanol/NH$_4$ Ac pH 6.9	319.17		HPLC-MS	[2]
	Zorbax SB-C_{18} (4.6 × 250 mm) methanol/NH$_4$Ac pH 6.9	319.17		HPLC-MS/MS	[3]
	FIA ACN/NH$_4$Ac (30:70)	319.17 160.09 $[M + 2H]^{2+}$	302.14 $[M-NH_3 + H]^+$	EC-ESI-TOF-MS	[25]
Trimeric compounds	Waters Atlantis ACN/ammonium formate pH 7.5	319		HPLC-MS/MS	[49]
Tetramer (−NH)	FIA ACN/NH$_4$Ac (30:70)	408.19		EC-ESI-TOF-MS	[25]
Tetramer (+O, −NH)	FIA ACN/NH$_4$Ac (30:70)	424.18		EC-ESI-TOF-MS	[25]
Pentamer	FIA ACN/NH$_4$Ac (30:70)	529.25 265.13 $[M + 2H]^{2+}$	512.23 $[M-NH_3 + H]^+$	EC-ESI-TOF-MS	[25]

ACN acetonitrile, *FIA* flow injection analysis, *MALDI* matrix-assisted laser desorption ionization, *NH$_4$Ac* ammonium acetate, *TFA* trifluoroacetic acid

and 6 [41]. It is more stable in apriotic solvents than in aqueous solutions.

The oxidation of PTD results in the formation of the analogous semitoluquinone diimine radical cation (STQDIRC; Table 3), for instance by reaction with bromine in acetate buffer. Michaelis et al. vaguely characterized it with UV–VIS spectroscopy (Table 5) [41]. Electrochemical oxidation combined with simultaneous UV–VIS

and EPR spectroscopic analysis led to an improved characterization of the chemical species [47].

para-Benzoquinone diimine

Similar to the SBQDIR cation, the formation of p-benzoquinone diimine (BQDI; Table 2) was detected by means of UV spectroscopy after oxidation of PPD with ferricyanide at pH 8 [35]. The diimine can be

preparatively produced [48]. Here, freshly prepared silver oxide was suspended in diethyl ether and then reacted with PPD whereby the solution developed a light yellow color. Interestingly, the diimine formation was used for a selective amperometric detection of PPD in hair dyes, as electrode material served glassy carbon modified with β-MnO_2 nanowires. At a redox potential of zero volt, a combined reversible chemical reaction and electron transfer process takes place at the electrode surface, where the diimine is oxidatively formed and reductively reconverted into the diamine. The reductive current served as a measure for the PPD concentration [36]. UV spectra substantiated the molecular structure of the BQDI, exhibiting a pink color. It preferentially forms under alkaline conditions. Recent studies tried to confirm the diimine formation by LC-MS/MS analysis. For instance, Aeby et al. supposed that a MS signal with the mass/charge (m/z) ratio of 107 amu together with the derived fragments m/z 80 amu and m/z 53 amu can be assigned to BQDI formed during PPD oxidation with ferricyanide in aqueous solution (Table 6) [49]. The same MS signal was recorded after electrochemical PPD oxidation in an amperometric cell. Furthermore, the diimine formation was indirectly ascertained by the determination of an adduct with a mass increment of 107 amu being formed by in situ electrochemical transformation of PPD in the presence of glutathione and cysteine [25].

The corresponding toluquinone diimine of PTD (Table 3) was UV/VIS spectroscopically determined in aqueous solution at pH 4 and 7 after electrochemical PTD oxidation [47]. The absorption maxima of 252 and 326 nm at pH 2 are comparable with that of the corresponding PPD-diimine. The oxidation and reduction potentials of the PTD and PPD diimines, measured by cyclic voltammetry, hardly differ. They are equally influenced by pH shifts.

para-Benzoquinone
Several authors supposed the generation of *p*-benzoquinone (PBQ; Table 2) from PPD under oxidation conditions relevant for the formation of azo (hair) dyes, but a reliable proof of the assumption is missing [3, 16, 49, 50]. It is hypothesized that PBQ is formed during hydrolysis of the corresponding diimine [51]. Presumably, the *p*-benzoquinone monoimine occurs as an intermediate of the reaction proceeding under alkaline conditions [52]. Due to the very small differences of UV/VIS spectra taken from solutions assumed to contain either PBQ or one of the corresponding imines, mainly this spectroscopic technique does not deliver sufficiently specific data for a sound identification of the questioned molecular structures.

There are a few studies conducted under specific reaction conditions, which report the almost complete transformation of PPD into PBQ. Ramachandran et al. described the

oxidation of PPD by Mn(III) acetate in sulfuric acid leading to Mn(II) ions and *p*-benzoquinone in the stoichiometric ratio of 2:1 [53]. The reaction product was extracted with diethyl ether and identified by UV and IR spectroscopy. Ignaczak et al. reported the transformation of PPD and PTD into the corresponding quinones by oxidation with Ce(IV) ions in perchloric acid [54]. Probably, the reaction proceeded via the hydrolysis of the intermediate semiquinone diimine.

para-Nitroaniline
Only one study stated the detection of *p*-nitroaniline (Table 2) [55]. The oxidation of PPD with hydrogen peroxide in weakly alkaline solution resulted in the formation of insoluble products. The precipitate was isolated and dissolved in dimethyl formamide (DMF), and the solved components were separated by thin layer chromatography (TLC). A spot whose quantity was dependent on the concentration of the oxidant could be assigned to *p*-nitroaniline. This compound was identified by means of the chromatographic R_f value, the melting point, and the IR spectrum. An analogous oxidation product of PTD is not known yet.

4-Aminophenyl formamide
When examining the biotransformation of PPD/d^4-PPD in reconstructed human epidermis with LC-MS, Nohynek et al. found unknown substances with m/z 137 and 141 amu (dosage of d^4-PPD), respectively [8]. A structure hypothesis for this compound was not formulated. They noticed though that this substance only occurred at high concentrations of PPD (>250 μM). Since it was detected in the pure medium, too, it was supposed to be an abiotically formed oxidation product of PPD. Based on MS/MS analysis, Hu et al. identified this unknown as 4-aminophenyl formamide (Table 2) [56]. After a 24-h exposition of human epidermis to PPD, they found a further substance with m/z 179 or 183 (d^4-PPD) amu, respectively, which they assigned to the molecular formula $C_9H_{10}N_2O_2$ and specified it as *N*-acetylated aminophenyl formamide. The acetylated metabolite was formed in the presence of the epidermis only.

Dimeric products
Dimeric compounds are generated either by the reaction (autoxidation) of two molecules of PPD or PTD or by the reaction of one of the aryldiamines with one of its oxidation products.

4,4'-Diamino azo benzene
Besides *p*-nitroaniline, the TLC-separation of PPD oxidation products by Dolinsky et al. led to the identification of another spot as 4,4′-diamino azo benzene (DAB; Table 2) [55]. Elemental analysis, thin layer chromatographic R_f

value, melting point, UV–VIS, and IR spectra served to characterize the compound. The identity of the substance was further proven by comparison with a standard synthesized via oxidation of *p*-aminoacetanilide. The oxidation of PPD with MnO_2 in anhydrous benzene resulted in a 30 % yield of the azo benzene [56]. The authors used the same separation and analytical techniques as Dolinsky [55].

Elsenberg applied HPLC in combination with diode array detection (DAD) and MS to analyze products of the PPD oxidation in aqueous H_2O_2 solution [57]. Using the standard addition method, he was able to identify DAB with improved analytical certainty. Recently, the formation of the dimeric derivative was confirmed by an electrochemical oxidation of PPD in a cell coupled online to an HPLC-MS system [25].

2-(4'-Aamino aniline)-1,4-diamino benzene
Electrochemical studies on solid electrodes with different techniques (chronopotentiometry, coulometry, rotating disk electrodes) revealed that an 1,4-addition can occur during the oxidation of PPD leading to the formation of 2-(4′-amino aniline)-1,4-diamino benzene (Table 2) [27, 58]. The reaction yield is strongly pH-dependent.

2-(4'-Amino aniline)-5-hydroxy-1,4-benzoquinone diimine
Dolinsky et al. detected this benzoquinone diimine (Table 2) after oxidation of PPD under alkaline conditions and TLC separation of the products [55]. The substance was identified by comparison of its IR, nuclear magnetic resonance (NMR), and UV–VIS spectra with those of a standard. Reduction resulted in the conversion into a colorless leuco base being reoxidized by air. The diimine also forms during the H_2O_2 oxidation of an equimolar mixture of PPD and hydroxy-PPD.

2-(1',4'-Benzoquinone diimine)-4-amino aniline and 2-(1',4'-benzoquinone diimine)-4-benzoquinone diimine
Both structure-related dimeric reaction products (mass difference: 2 amu) 2-(1′,4′-benzoquinone diimine)-4-amino aniline and 2-(1′,4′-benzoquinone diimine)-4-benzoquinone diimine (Table 2) were formed during electrochemical PPD oxidation and identified by HPLC-ESI-TOF-MS. The formation of both products depended on the oxidation potential. Both compounds formed adducts with glutathione and cysteine, identified by the same MS technique (Table 6) [25].

Dimeric products of PTD
The structural elucidation of dimeric oxidation products of PTD is more challenging than analyzing PPD derivatives, since a determination of the ring positions of the methyl groups is required additionally. In the last years, only Goux et al. made efforts in this direction [47]. The spectroelectrochemical oxidation of PTD on a platinum

electrode at neutral pH resulted in the formation of a dimer proven by UV/VIS and EPR spectra (Table 3). The compound did not form at pH 2 and 10. The molecular structure could not be precisely defined, since the position of the methyl groups could not be clarified with the applied analytical techniques. Recently, Fischer et al. detected UV-photolysis products of PTD by means of HPLC-ESI-MS. Chromatographic retention times and molecular masses of 241.2 and 242.2, respectively, would be consistent with dimeric structures [26]. A further characterization of these compounds remains outstanding.

Trimeric reaction products
Bandrowski's Base
In 1894, Bandrowski already described a trimeric PPD derivative obtained by the oxidation of PPD with oxygen or ferricyanide in ammoniacal solution [24]. Later on, this compound was named after him (Table 2). The molecular formula deduced from the elemental analysis gave no clue about the precise molecular structure. Various suggestions about the structure were made. Reproducing the findings of Bandrowski, several investigations, conducted in the 1960s, revealed improved structural information, based on a combination of physico-chemical, chromatographic, and spectroscopic, e.g., NMR, data [55, 59–62]. Corbett hydrolyzed the trimeric compound and yielded 2,5-dihydroxy benzoquinone, identified by its UV/VIS absorbance spectrum [62]. Further evidence for BB formation during PPD oxidation by H_2O_2 was attained by Bracher et al. who performed Ames tests with mixtures of PPD oxidation products, formed in the presence and absence of couplers, i.e., resorcinol [63]. The trimeric product, formed in ammoniacal solution in the absence of resorcinol, was isolated by TLC and exhibited the same properties as a BB standard. BB also forms during irradiation of a PPD solution in dimethyl sulfoxide (DMSO) [64]. Its structure was examined after TLC isolation by UV–VIS and IR spectroscopy. Novel analysis with HPLC-DAD and HPLC-MS confirmed the formation of BB during reaction of PPD in ammoniacal solution in the presence of the oxidants oxygen, ferricyanide, or hydrogen peroxide [25, 49, 57].

Goux et al. determined an analogous product of PTD by spectroelectrochemical methods (cyclic voltammetry-UV/VIS spectroscopy) in the pH range of 4 to 7 (Table 3) [47]. The structural evidence is merely based on the comparison of the obtained UV spectra with that of a BB standard. Fischer et al. tentatively identified a compound with a molecular mass of 343.3 amu as a trimeric PTD derivative formed during UV-photolysis of aqueous PTD solutions [26].

Azine
The intramolecular rearrangement of BB leads to azine (Table 2), which can be defined as the leuco form of

trimeric *p*-amino diphenylamine also [65–69]. Quinone-like products arise by further reactions of azine under alkaline conditions. Polymeric compounds are generated by the reaction of azine with PPD molecules. Li et al. synthesized polymers with azine as the basic structural unit by controlled polymerization of PPD [69].

Further oligomeric and polymeric compounds
Besides various dimeric and trimeric products, the electrochemical transformation of PPD resulted in several oligomers with higher molecular masses, investigated on-line with a coupled ESI-MS-TOF system. Some of the detected ion masses were attributed to tetramers and pentamers and to their fragmentation products [25]. Based on the current knowledge regarding the reactivity of the interesting aryldiamines, it can be surmised that the oxidation reactions proceed via the formation of a plethora of oligomeric and polymeric interim products, differing in number, type, and steric arrangement of their monomeric building blocks.
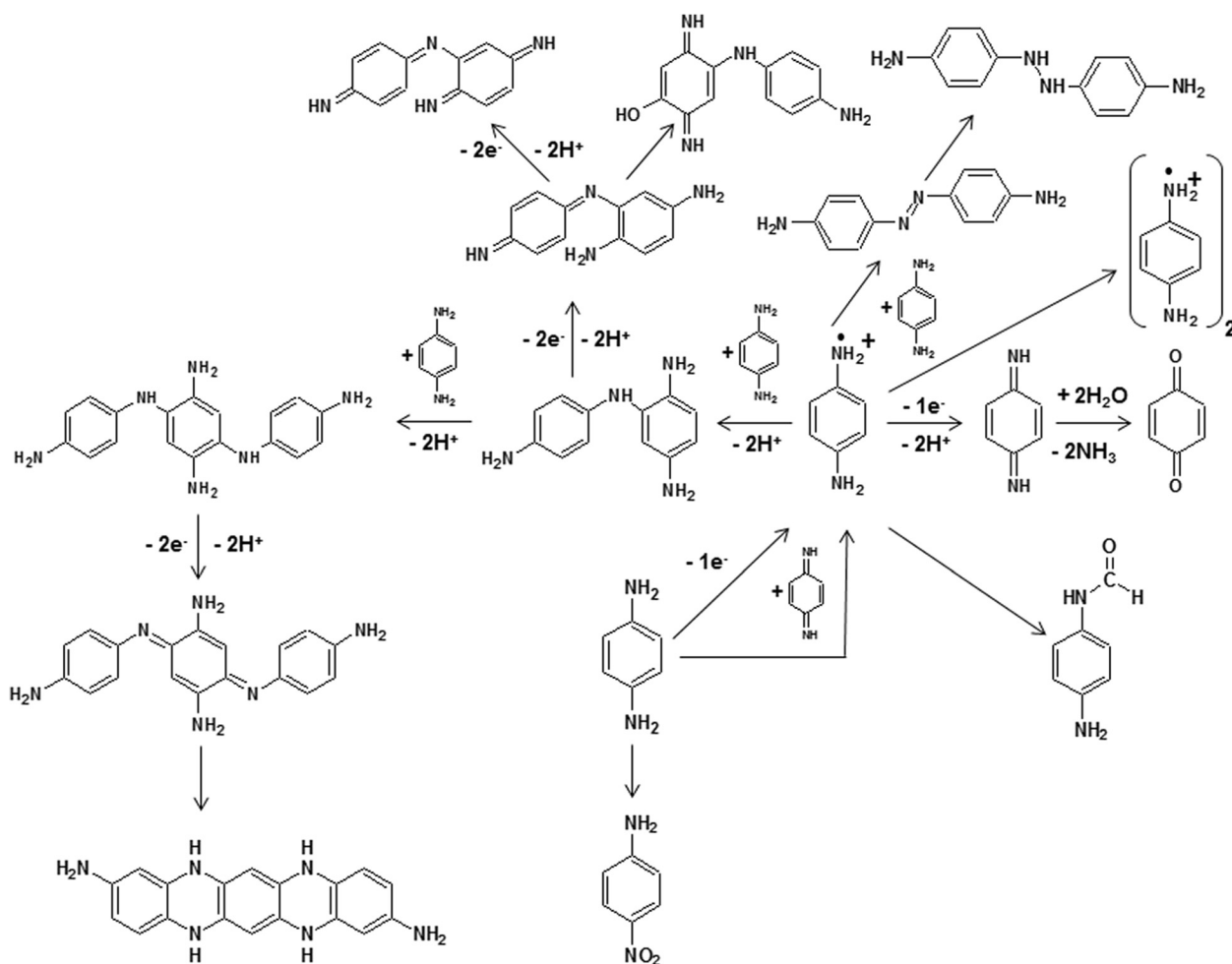
Reaction pathways of PPD and PTD
The following chapter aims at an assembling of the main aryldiamine transformation products into an integrated reaction scheme, focusing on PPD (Fig. 1). Particular PTD transformation pathways are only mentioned in cases where an analytical confirmation of specific reaction steps and products exists. The analytical work substantiating the process scheme is not referred again.

Reaction pathways of PPD
Monomeric products A central step in the transformation of PPD is the BQDI formation via radical reaction. Two pathways lead to BQDI generation: a) direct formation without interim products via a two-electron transfer (Scheme 1) b) formation by two one-electron steps via the intermediate SBQDIRC.

Indications exist for a pH-depending equilibrium between SBQDIR and its protonated form, the SBQDIR cation. Cyclic voltammetry is able to ascertain the reversibility of redox processes and it allows the determination of redox

Fig. 1 Reaction pathways of PPD
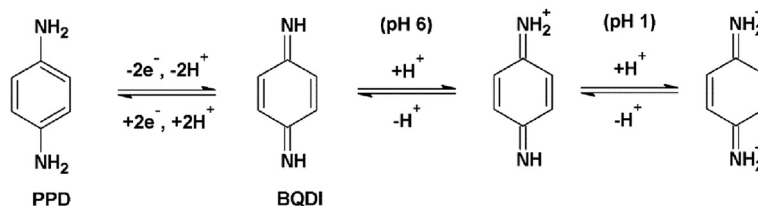
Scheme 1 BQDI formation from PPD via a two electron transfer

potentials. Thus, this technique together with EPR is mainly used to clarify the electron transfer mechanisms of PPD. In aqueous solutions, a two-electron transfer process takes place under electrochemical oxidation conditions mainly. Using various electrodes, it was shown that the PPD oxidation was reversible independently from the applied electrode material [36, 38, 69, 70]. The reaction proceeds according to the one-electron transfer mechanism when a potential higher than the standard PPD oxidation potential is applied to the electrodes. In addition, this mechanism is important for the PPD oxidation in organic solvents as well as for transformation processes initiated by various chemical oxidants [43]. The intermediately formed SBQDIRC has a half-life of about 2.5 s in neutral aqueous solutions. The stability of the radical increases with acidification. Longer radical lifetimes were ascertained in organic solvents, e.g., methanol [41].

The components PPD, BQDI, and SBQDIR form the following reaction equilibrium [71]:

$$PPD \;+\; BQDI \;\rightleftarrows\; 2\,SBQDIR \tag{1}$$

An important side reaction of BQDI is polymerization [69]. Since protonation stabilizes BQDI, the polymerization velocity increases with increasing pH [72]. PBQ is assumed as a further side reaction product of BQDI. A proof of this hypothesis is missing at least for toxicologically or environmentally relevant reaction conditions. It is supposed that the reaction progresses via a nucleophilic substitution of one of the both imine groups by oxygen, leading to the intermediate benzoquinone monoimine (BQMI). Its formation is not proven as well. Indications for this reaction pathway were established by Tong who studied the velocity of the deamination of *N,N'*-dialkyl-aryldiamines [52].

Dimeric products
The oxidation of PPD in benzene by MnO_2 yields DAB mainly. Bhatnagar and George postulated a dimerization of the intermediately formed SBQDIR(C) into a hydrazine, subsequently oxidized to DAB [73]. Another reaction pathway proceeds via the nucleophilic substitution of PPD by SBQDIR(C) in ring position 2, yielding 2-(1′,4′-benzoquinone diimine)-4-amino aniline (Table 2). In the next step,

this molecule is oxidized to 2-(1′,4′-benzoquinone diimine)-1,4-benzoquinone diimine [25].

Trimeric products
The main pathway leading to trimeric products is the nucleophilic attack of PPD or SBQDIR(C) on one of the dimeric compounds. Several studies demonstrated that BB is only formed in the absence of couplers, e.g., resorcinol, hydroquinone, or aminophenol. A redox equilibrium of BB with its reduced form exists.

Reaction pathways of PTD
A few studies were conducted to elucidate the oxidative transformation of PTD. With respect to the structural and electronic relationship to PPD, a comparable reactivity of PTD, i.e., analogous reaction pathways and products, can be expected. Nonetheless, the additional methyl group might influence the reaction behavior of PTD in a number of aspects:

a) Increase of the electron density of the delocalized π-electron system due to the positive inductive effect of the methyl group. Compared to PPD, the velocity of nucleophilic aromatic substitution reactions should be reduced, the electrophilic substitution should be accelerated.
b) One ring position is blocked, resulting in lower rates of ring attacking reactions.
c) In contrast to PPD, the H-atom bearing ring positions are not electronically equivalent. The methyl group has a directing effect on the positioning of entering substituents depending on the reaction type and its electronic transition state.
d) Ring attachment of substituents results in the formation of different stereoisomers.
e) The methyl group itself might be targeted by oxidation reactions.

According to an early investigation, a SBQDIRC-analogous compound, the STQDIRC, is formed by the action of bromine on PTD in an acetate buffer/methanol solution [41]. Recently, the occurrence of this radical was confirmed, and the pH dependence of its generation and reactivity was determined based on

combined electrochemical (cyclic voltammetry) and in situ spectroscopic (EPR and UV/VIS absorbance) measurements [47]. The radical has a lifetime of a few hours at neutral pH, but it reacts much faster at low and high pH. Subsequently, the toluquinone diimine (TQDI) is formed which attacks a PTD molecule to yield a dimeric product, not further characterized yet. Analogously to PPD, trimeric products including a BB-like structure originate from the reaction of dimeric intermediates with STQDIRC or TQDI.

Intrinsic difficulties of process and product elucidation

Investigating reaction pathways and products of aryldiamines, one has to cope with some fundamental difficulties. First of all, the reaction of aqueous aryldiamine solutions with oxygen or oxygen-containing oxidants is able to proceed during several days with increasing yield of oligomeric and polymeric compounds. It is not clear whether a distinct reaction equilibrium can be practically reached and how the corresponding product spectrum will be composed of. Only a few dimeric and trimeric compounds, e.g., DAB and BB, seem to be relatively stable. Consequently, most of the detected products are intermediates whose lifetimes are highly variable and regulated by factors widely unknown. Due to the low selectivity of involved radical reactions, it can be assumed that the number of potential reaction products rises with the increasing degree of oligomerization of the attacked intermediates almost exponentially, forming a reaction cascade. Additionally, the whole reaction system seems to comprise autocatalytic steps including variable induction periods and feedback loops making it extremely sensible against smallest differences in substance composition, e.g., stoichiometric aryldiamine to oxidant ratio, content of impurities, spatial distribution of reactants in the reaction vessel, and against changes of physical reaction conditions, e.g., temperature and eventually radiation. Especially, the equilibrium between PPD, SBQDIR, and BQDI seems to be very easily influenceable [74]. Hints for autocatalytic reaction steps were deduced from examinations of the correlation between oxygen consumption and BB formation at pH values >8.5 [75]. Thus it can be inferred that the reproducibility of determined product composition is very low. This conclusion was firstly expressed by Nickel at al. who studied the photographic color development process, based on the oxidation of PPD by Ag^+ ions [74, 76]. They assumed the existence of many finalized yet unpublished investigations of PPD oxidation under the relevant slightly acidic conditions and they explained this situation with an insufficient reproducibility of measurements. None of the cited publications pay particular attention to the reproducibility of the results; mostly this topic is not mentioned at all.

If autocatalytic reaction steps take place, the time-dependent product composition depends on the homogeneity of the distribution of all reaction factors, e.g., reactants, temperature, and light, in the reaction vessel. The dimension of the latter becomes a critical factor as well as its material composition, surface area, and surface properties. Up to now, no study has addressed these points.

There are various analytical challenges as well. Several of the intermediates are assumed to have short lifetimes and to be formed in low concentrations. Their isolation is difficult if ever possible. If the reaction mixtures are chromatographically separated, e.g., by HPLC, one has to consider unwanted and uncontrollable reactions of reactive intermediates with components of the analytical device producing analytical artifacts.

Conclusions

Despite of more than 100 years of research the understanding of (aut)oxidation processes of PPD and PTD is fragmentary. Many reaction products are not or not unambiguously identified. According to Table 2, only the formation of SBQDIR(C), BQDI, DAB, and BB seems to be sufficiently confirmed. None of the PTD reaction products is satisfactorily characterized. For this, the reasons are manifold. Some belong to the above mentioned intrinsic complexity of the reaction. Others originate from an insufficient control or documentation of the reaction conditions strongly limiting the comparability of independent studies. Older investigations are handicapped by the lower performance of the applied separation and detection techniques. Generally, the evaluation of analytical data is impeded by the lack of required reference chemicals.

High performance analytical instruments like quadrupole-TOF-MS or orbitrap MS offer new analytical capabilities in terms of detection sensitivity, mass accuracy, generation of characteristic fragmentation patterns, and speed. These devices are suited for online coupling with reaction vessels allowing for kinetic measurements with high time resolution. To minimize residence time and reactive interactions in separation systems, direct sample injection is an option. If separation cannot be circumvented, fast (U)HPLC techniques, applying microbore or capillary columns, are available. Capillary electrophoresis might be a promising alternative to chromatographic techniques, still unexplored for this task. Since a reliable substance identification requires two powerful and independent analytical methods, at least the application of NMR techniques seems to be essential. Obstacles are the relatively low NMR detection sensitivity and the need for relatively pure samples. The last point can be addressed by HPLC-NMR.

A promising development is the combination of electroanalytical methods, i.e., voltammetry, with in situ spectroscopic investigation. SERS is a powerful complementary

spectroscopic technique if the electrodes are able to exert the SERS effect.

All of these analytical reflections end up with considerable experimental and instrumental efforts. It might be questionable whether these expenditures might be reasonable beyond pure academic interests, since several studies have proven that certain toxic reaction products, e.g., BB, are not formed by the proper application of PPD containing personal care products. Nevertheless, the various regulations and limitations of the use of PPD in personal care products are not substantiated by the intrinsic toxicity of PPD only but by suppositions regarding harmful reaction products also. To provide a sound basis for an improved risk assessment for PPD and related aryldiamines as well as for further regulative decisions, further efforts to uncover their reaction pathways and products formed under human exposition conditions seem to be inevitable.

Abbreviations

BB: Bandrowski's Base; BfR: German Federal Institute for Risk Assessment; BQDI: benzoquinone diimine; BQMI: benzoquinone monoimine; CIR: Cosmetic Ingredient Review; CVA: cyclic voltammetry; DAB: diamino azobenzene; DAD: diode array detector; DFG: German Research Foundation; DMF: dimethyl formamide; DMSO: dimethyl sulfoxide; EECCD: European Economic Community Cosmetics Directive; EPR: electron paramagnetic resonance spectroscopy; ESI: electrospray ionization (MS interface); FDA: Food and Drug Administration; FIA: flow injection analysis; GCE HPLC: glassy carbon electrode HPLC high performance liquid chromatography; IARC K_{ow}: International Agency for Research on Cancer Kow octanol—water distribution coefficient; MALDI: Matrix-assisted laser desorption ionization; NMR: nuclear magnetic resonance spectroscopy; ox. ag.: oxidizing agent; PBQ pK_a: *para*-Benzoquinone acidity pKa constant; PPD: *para*-Phenylenediamine; PTD: *para*-Toluenediamine; SBQDIRC: semibenzoquinone diimine radical cation; SCCNFP: Scientific Committee on Cosmetic Products and Non-Food Products; SCCP: Scientific Committee on Consumer Products; SERS: Surface enhanced Raman spectroscopy; solv.: solvent; STQDIRC: semitoluquinone diimine radical cation; TFA: trifluoroacetic acid; TLC: thin layer chromatography; TOF: time-of-flight (MS technique); TQDI: toluquinone diimine.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Both authors contributed equally to this work. Both authors read and approved the final manuscript.

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