Paracetamol in the environment and its degradation by microorganisms

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Abstract Paracetamol (4'-hydroxyacetanilide, *N*-acetyl-*p*-aminophenol, acetaminophen, and paracetamol) is a widely used over-the-counter analgesic and antipyretic drug. Paracetamol and structural analogs are ubiquitous in the natural environment and easily accumulate in aquatic environment, which have been detected in surface waters, wastewater, and drinking water throughout the world. Paracetamol wastewater is mainly treated by chemical oxidation processes. Although these chemical methods may be available for treating these pollutants, the harsh reaction conditions, the generation of secondary pollutants, and the high operational cost associated with these methods have often made them not a desirable choice. Biodegradation of paracetamol is being considered as an environmentally friendly and low-cost option. The goal of this review is to provide an outline of the current knowledge of biodegradation of paracetamol in the occurrence, degrading bacteria, and proposed metabolic/biodegrading pathways, enzymes and possible intermediates. The comprehensive understanding of the metabolic pathways and enzyme systems involved in the utilization of paracetamol means will be helpful for optimizing and allowing rational design of biodegradation systems for paracetamol-contaminated wastewater.

Keywords Paracetamol (acetaminophen) · Structural analogs · Metabolic/biodegrading pathway

Introduction

Pharmaceuticals and their metabolites are subclasses of organic contaminants that have been detected in wastewater and surface waters throughout the world (Benotti et al. 2009; Philips et al. 2010). These emerging pollutants are continuously introduced into the aquatic environment by several emissions from manufacturing facilities, consumer use and disposal, and hospital waste (Langford and Thomas 2009; Thomas and Langford 2007; Philips et al. 2010). Even though the detected concentrations are typically in the nanogram- to microgram-per-liter range, it cannot be excluded that molecules designed to be biologically active affect sensitive aquatic organisms even at such low concentrations (Huber et al. 2005). A variety of potential adverse effects, including acute and chronic damage, accumulation in tissues, reproductive damage, inhibition of cell proliferation, and behavioral changes, have been documented in these low levels (Philips et al. 2010; Escher et al. 2011). Currently, very little data exist on the levels of pharmaceuticals in the environment.

The most prevalent pharmaceuticals in treated wastewater are typically those that are most frequently prescribed or purchased over the counter, such as a series of substituted acetanilides including paracetamol and structural analogs or their possible intermediates. Paracetamol, also known as 4-acetaminophenol, IUPAC name *N*-(4-hydroxyphenyl)ethanamide, is a very common over-the-counter analgesic used for fever, headaches, and other minor pain. Paracetamol consists of a benzene ring core substituted by one hydroxyl group and the nitrogen atom of an amide group in the *para* (1,4) pattern (Bales et al. 1985). As part of the class of drugs known as “aniline analgesics,” paracetamol is the only such drug still in use today. Paracetamol and structural analogs are depicted in Fig. 1.

The pharmacological effects of paracetamol are generally considered to be based on inhibition of prostaglandin synthesis (Malmberg and Yaksh 1982). In fact, the mechanism of

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Fig. 1 Paracetamol and structural analogs

action of paracetamol is not completely understood till now. The main mechanism proposed is the inhibition of cyclooxygenase, and recent findings suggest that it is highly selective for COX-2. At therapeutic levels, paracetamol is considered to be safe for humans upon normal drug use (Thomas 1993). The absorption of low therapeutic doses of paracetamol is usually rapid and complete; the systemic bioavailability and the plasma half-life being about 75 % at 1.5 to 2.5 h, respectively.

Frequent occurrence of paracetamol in aquatic environments and drinking water has raised a concern about their potential effects on environment and human health. Paracetamol is ranked as one of the top three drugs prescribed in England and is one of the top 200 prescriptions in the USA (Sebastine and Wakeman 2003; Zhang et al. 2008). In 2002, the USA produced 3.6×10^9 g of paracetamol (Bedner and Maccreehan 2006). In 1998, it was estimated that 3.2×10^9 tablets were consumed in the UK alone (Bessemers and Vermeulen 2001). Paracetamol and their metabolites are continuously introduced into the aquatic environment by consumer use and disposal and hospital waste (Langford and Thomas 2009; Philips et al. 2010). Even paracetamol that is not highly persistent in the environment can be found at elevated concentrations and causes negative ecological effects because its continuous introduction into the environment can overwhelm its high transformation rates (Petrovic et al. 2003). 4-Aminophenol is the hydrolytic product of acetaminophen

(paracetamol) and is reported to have significant nephrotoxicity and teratogenic effects and has been detected as a synthetic intermediate (Sumera et al. 2006). Muir et al. (1997) have demonstrated that 58–68 % of paracetamol and their metabolites are excreted from the body during therapeutic use.

Numerous studies have demonstrated that microorganisms play the major roles in the paracetamol degradation in the environment under various conditions, which has been reported by Hu et al. (2012). This paper is focused on summarizing the studies on paracetamol biodegradation, mainly in the last decade, in the following aspects: paracetamol in the occurrence, degrading bacteria, and proposed metabolic/biodegrading pathways in animals or microorganisms, enzymes, and possible intermediates.

Paracetamol in the environment

Paracetamol consumption throughout the world has increased. In the Nordic countries, usage increased fivefold between 1978 and 1988, and in 1994–1995, the rates in some developed countries exceeded 20 g/person/year (Sheen et al. 2002). Paracetamol was dispensed in 2006 in Wales (population approximately three million) at the level of over 140 tons, more than 45 g/person/year (Jones et al. 2007; Verlicchi et al. 2010). In the UK, approximately $3.2 \times$

10^9 tablets of paracetamol are consumed every year, which is an average of 55 tablets/person (Jones 1998). Paracetamol are easily accumulated in aquatic environment due to their high solubility and hydrophilicity, which have been detected in surface waters, wastewater, and drinking water throughout the world. Paracetamol is known to occur at high microgram per liter concentrations in influents and is, for the most part, effectively eliminated by wastewater treatment plants (WWTPs) (Thomas et al. 2007). It has been found with a concentration of up to $6 \mu\text{g L}^{-1}$ in European sewage effluents (Ternes 1998), up to $10 \mu\text{g L}^{-1}$ in US natural waters (Kolpin et al. 2002), and more than $65 \mu\text{g L}^{-1}$ in Tyne River in the UK (Robert and Thomas 2006). In hospital effluents and WWTPs influents, paracetamol concentrations can exceed $150 \mu\text{g L}^{-1}$; however, effective elimination by WWTPs often results in low nanogram per liter concentrations being detected in WWTPs discharges. In a 2002 survey of 139 US streams, Kolpin et al. (2002) detected acetaminophen in roughly 25 % of the samples tested at a median concentration of $0.11 \mu\text{g L}^{-1}$. The median concentration of paracetamol detected in surface waters is $0.055 \pm 0.051 \mu\text{g L}^{-1}$ (Wiegel et al. 2004; Bound and Voulvoulis 2006; Gros et al. 2006). In raw wastewater, paracetamol was detected at a median concentration of $48 \pm 75 \mu\text{g L}^{-1}$ (Han et al. 2006; Gomez et al. 2007). The median concentration of paracetamol detected in finished wastewater is $0.76 \pm 0.96 \mu\text{g L}^{-1}$ (Gros et al. 2006; Han et al. 2006; Gomez et al. 2007).

The widespread use of acetaminophen raises the concern of whether or not the compound persists during treatment of wastewater and drinking water. One of the most common treatment processes in water utilities is chlorination. Bedner and Maccreehan (2006) reported that, during chlorination of acetaminophen, 11 different chlorination products were observed, including the toxic substances *N*-acetyl-*p*-benzoquinone imine (NAPQI) and 1,4-benzoquinone. Although these toxicants may exist only at very low levels in drinking water and wastewater, their presence along with multiple other pharmaceuticals deserves further consideration (Bedner and Maccreehan 2006). Table 1 summarizes the occurrence

of paracetamol and the range of concentrations detected in water samples.

Biodegradation of paracetamol by pure culture

Nowadays, paracetamol wastewater is mainly treated by chemical oxidation processes such as electrochemical (Brillas et al. 2005), ozonation (Andreozzi et al. 2003), $\text{H}_2\text{O}_2/\text{UV}$ oxidation (Andreozzi et al. 2003; Skoumal et al. 2006), TiO_2 photocatalysis (Yang et al. 2008, 2009), and solar photoelectro-Fenton oxidation (Li et al. 2007). Although these chemical methods may be available for treating these pollutants, the harsh reaction conditions, the generation of secondary pollutants, and the high operational cost associated with these methods have often made them not a desirable choice (Li et al. 2007; Chen et al. 2010). Biodegradation of pharmaceutical compounds is being considered as an environmentally friendly and low-cost option and has been demonstrated to have the potential to eliminate pharmaceuticals by degrading them into innocuous end products such as CO_2 and H_2O (Hasan et al. 2011; Chen et al. 2010). However, little information is available about the biodegradation of paracetamol compared to other pharmaceutical compounds.

A mould which was isolated from a solution of paracetamol was identified as a *Penicillium* species and was found to possess the ability to utilize a series of substituted acetanilides, including paracetamol (4-hydroxyacetanilide), phenacetin (4-ethoxyacetanilide), and metacetamol (3-hydroxyacetanilide) as sole carbon sources for growth (Hart and Orr 1975). Microorganisms have established effective strategies involving specialized enzyme systems and metabolic pathways to access paracetamol as a carbon and energy source. Thus, such microorganisms are capable of degrading paracetamol and converting them to easily metabolizable substrates. In addition, in-depth characterization of genes and enzyme systems involved in the utilization of paracetamol has led to an improved understanding of microbial paracetamol metabolism. A large number of

Table 1 Paracetamol occurrence in the environment (in micrograms per liter)

Surface water	Raw wastewater	Finished wastewater	Finished drinking water
<0.1 (Stackelberg et al. 2004)	0–0.26 (Han et al. 2006)	0–6.0 (Ternes 1998)	Low ppb levels (Han et al. 2006)
0.11 (Kolpin et al. 2002)	29–246 (Gomez et al. 2007)	0–0.16 (Han et al. 2006)	
0–0.026 (Boyd and Furlong 2002)	0.13–26 (Gros et al. 2006)	1.9 (Brun et al. 2006)	
0–0.25 (Gros et al. 2006)	1.2–10 (Kolpin et al. 2002)	0–4.3 (Gomez et al. 2007)	
0.52–0.56 (Bound and Voulvoulis 2006)	22–65 (Robert and Thomas 2006)	0–5.99 (Gros et al. 2006)	
0.007–0.066 (Wiegel et al. 2004)	6 (Ternes 1998)	0.048–0.418 (Radjenovic et al. 2007)	
	10 (Kolpin et al. 2002)	0.079–0.22 (Bound and Voulvoulis 2006)	

Table 2 Bacterial strains capable of utilizing paracetamol as a sole carbon source

Species, strain	Isolated from	Concentration (mgL ⁻¹)	Performance	References
<i>Stenotrophomonas</i> sp. f1	Paracetamol-degrading aerobic aggregate	400	Completely degradation in 116 h	Zhang et al. (2012)
<i>Pseudomonas</i> sp. f2	Paracetamol-degrading aerobic aggregate	2,500	Completely degradation in 70 h	Zhang et al. (2012)
<i>Pseudomonas</i> sp. fg-2	Paracetamol-degrading aerobic aggregate	2,000	Completely degradation in 45 h	Zhang et al. (2012)
<i>Delftia tsuruhatensis</i>	In a membrane bioreactor biomass	100	Shaking flask, 97 % degraded in 48 h	Bart et al. (2011)
<i>Pseudomonas aeruginosa</i>	In a membrane bioreactor biomass	16	Shaking flask, 40 % degraded in 48 h	Bart et al. (2011)
<i>Penicillium</i> species	An acidic solution of paracetamol	0.01 % (w/v)	Shaking flask, 98 % degraded in 36 h	Hart and Orr (1975)
Strain P1(unidentified)	An enrichment cultured consortium	1,000	96.3 % degraded in 20 h	Xu et al. (2003)
<i>Cupriavidus necator</i> F1	Activated sludge samples	400	Completely degradation in 48 h	Wei et al. (2011)
<i>Burkholderia</i> sp. AK-5	Rice field soil (4-aminophenol)	2,140	Shaking flask, 99 % degraded in 15 h	Murakami et al. (2003)
<i>Pseudomonas</i> sp. ST-4	Activated sludge samples (4-aminophenol as substrates)	200 ppm	Shaking flask, 80 % degraded in 72 h	Sumera et al. (2006)
<i>Pseudomonas</i> sp. AP-3	Activated sludge samples (2-aminophenol as substrates)	340 ppm	Shaking flask, 70 % degraded in 48 h	Takenaka et al. (1997)

microorganisms belonging to the phyla of eubacteria, yeast, and fungi and also some algae capable of using paracetamol as carbon and energy source have been described. A listing of isolated bacterial strains characterized for the degradation of those paracetamols is presented in Table 2.

The utilization of paracetamol and phenacetin as nitrogen sources by *Penicillium* species has been studied by Hart and Orr (1975), while Betz and Clarke (1973) showed that strains of *Pseudomonas putida*, *Pseudomonas cepacia*, and *Pseudomonas acidovorans* could grow in a mineral salts medium with acetamide or phenylacetamide as the carbon or nitrogen source.

Bart et al. (2011) reported that two paracetamol-degrading strains were isolated from the MBR biomass and identified as *Delftia tsuruhatensis* and *Pseudomonas aeruginosa*. Three bacterial strains utilizing paracetamol as the sole carbon, nitrogen, and energy source were isolated from a paracetamol-degrading aerobic aggregate and assigned to species of the genera *Stenotrophomonas* and *Pseudomonas*. The *Stenotrophomonas* species have not included any known paracetamol degraders until now. In batch cultures, the organisms f1, f2, and fg-2 could perform complete degradation of paracetamol at concentrations of 400, 2,500, and 2,000 mg/L or below, respectively (Zhang et al. 2012). Zhang (2012) also reported that a combination of the three microbial strains recovered from the same culture was shown to be necessary for complete paracetamol degradation and mineralization, suggesting a possible complementary interaction among the various isolates.

Under aerobic conditions, *Pseudomonas* sp. is capable to utilize aromatic compounds as carbon energy

source (Ahmed et al. 1999). The versatility of *Pseudomonas* makes it an attractive organism for its use in waste treatment application where wide ranges of organic substances are present. The *Pseudomonas* sp.

Fig. 2 Main pathways of paracetamol metabolism in animals

strain ST-4 was adapted to different concentrations of 4-aminophenol from 50 to 500 ppm (Sumera et al. 2006). *Pseudomonas* strain AP-3 had been reported about growth on 2-aminophenol (Takenaka et al. 1997). Aromatic amines have always been related with the property of autoxidation. Actually, these compounds are highly sensitive towards light and temperature, and their continuous exposure to intense heat and light causes their oxidation. The rate of autoxidation was studied at different concentrations. The results showed that a very low rate of autoxidation occurred during the course of experiment. Evaporation and autoxidation are proved to be minor elimination mechanisms removing 1 % of the added aniline/day. Instantaneous binding to the humic components removed 4 %. Biodegradation was clearly the most significant removal method to eliminate aniline from aquatic environment. It had also been declared that, although the properties of the autoxidation do exist among the aromatic amines, but the rate was much lower than that of the degradation rate by microbes.

Proposed metabolic pathways, enzymes, and possible intermediates

During the past decades, research related to paracetamol biodegradation has focused on the identification and characterization of metabolic intermediates involved in the initial step of aerobic bacterial catabolic pathways. Most of the large amounts of investigations regarding metabolic pathways of paracetamol have of course been performed in (laboratory) animals. In animals, paracetamol is metabolized primarily in the liver into nontoxic products. Three metabolic pathways are notable: (1) glucuronidation is believed to account for 40 % to two thirds of the metabolism of paracetamol; (2) sulfation (sulfate conjugation) may account for 20–40 %; (3) *N*-hydroxylation and rearrangement, then GSH conjugation, account for less than 15 %. The hepatic cytochrome P450 enzyme system metabolizes paracetamol, forming a minor yet significant alkylating metabolite known as *N*-acetyl-*p*-benzoquinone imine. NAPQI is then irreversibly conjugated with the sulfhydryl groups of glutathione

Fig. 3 Proposed metabolic pathways of paracetamol in man and experimental animals. The individual metabolites were all detected in the urine of organisms that ingested paracetamol in therapeutic quantities (man) (*P450* cytochrome P450, *NAT* *N*-acetyltransferase, *ST* sulfotransferase, *UDPGT* uridine

(see Fig. 2). As Thomas reported, paracetamol is metabolized primarily by glucuronidation and sulfation. About 30 and 55 % of administered paracetamol (PAR) is excreted in urine as conjugates PAR-sulfate and PAR-glucuronide, respectively (Thomas 1993). Like in various laboratory animals, a small amount of PAR is probably metabolized via a third metabolic pathway, that is, oxidation by the microsomal cytochrome P450 (CYP)-containing mixed function oxidase system to NAPQI. A glutathione 1,4-Michael adduct of NAPQI and the corresponding cysteine conjugate and mercapturic acid breakdown products were found in human urine after ingestion of PAR (Prescott 1980). A composition of the metabolic pathways of paracetamol in man and experimental animals is depicted in Fig. 3.

With different animals, in most described cases, paracetamol conversion in microbes was proposed to proceed via 4-aminophenol to hydroquinone, which is the major route in biodegradation. Paracetamol is converted to the corresponding 1,4-benzenediol or hydroquinone compounds, i.e., hydroquinone pathway. The paracetamol is initially catalyzed by an amidohydrolase with the release of acetate to yield 4-aminophenol, in which the amino group is then replaced by a hydroxyl group to form hydroquinone, subsequent to ring fission (Fig. 4, steps 1 and 3). In the

second instance, the initial hydroxylation of paracetamol could potentially be catalyzed by a hydrolytic enzyme to produce hydroquinone with a release of acetamide, which could be further converted into oxamic acid (Zhang et al. 2012) (Fig. 4, step 2). However, pyrocatechol pathway has also been described by Wei et al. (2011). The identified metabolites suggested another possible main route for paracetamol metabolism (Wei et al. 2011). In the initial reaction, the paracetamol is oxidized by hydroxylase and yields the corresponding *N*-(4-hydroxyphenyl)-acetamide as the first metabolites in which acetyl group was replaced by hydrogen atom to form *p*-aminophenol (Fig. 4, step 5), and then the *N*-(4-hydroxyphenyl)-acetamide is oxidized to the pyrocatechol by substrate-specific oxygenase and formamide CGTase (Fig. 4, steps 8 and 10). Another route may be similar to hydroquinone pathway. Subsequent oxidation of *N*-(4-hydroxyphenyl)-acetamide leads to the formation of the hydroquinone and 1,4-benzoquinone that is subsequent to ring fission substrate *p*-phenol, which finally participated in TCA cycles (Fig. 4, steps 6 and 9). Hydroquinone and pyrocatechol pathways were proposed as Fig. 4. *Burkholderia* sp. strain AK-5 could possibly transform 4-aminophenol to 1,2,4-trihydroxybenzene via 1,4-benzenediol. 1,2,4-Trihydroxybenzene 1,2-dioxygenase cleaved the benzene ring

Fig. 4 Proposed pathway for biodegradation of paracetamol and the enzymes involved. 1 amidohydrolase, 2 hydrolytic enzyme, 3 amino acid oxidase, 4 monooxygenase, 5 hydroxylase, 6 amidohydrolase, 7 hydrolytic enzyme, 8 oxygenase, 9 laccase, 10 formamide CGTase

Fig. 5 Proposed pathway of 4-aminophenol metabolism in *Burkholderia* sp. strain AK-5 and comparison to the metabolic pathways of aniline and aniline derivatives. **a** Proposed pathway of 4-aminophenol metabolism; **b** metabolic pathways of aniline and aniline derivatives in *P. putida* 2NP8; **c** proposed reaction pathways during photocatalytic degradation of paracetamol

of 1,2,4-trihydroxybenzene to form maleylacetic acid (Murakami et al. 2003). Figure 5a shows the proposed metabolic pathway of 4-aminophenol in strain AK-5. 4-Aminophenol was converted to 1,2,4-trihydroxybenzene via 1,4-benzenediol; 1,2,4-trihydroxybenzene 1,2-dioxygenase catalyzed the conversion of 1,2,4-trihydroxybenzene to

maleylacetic acid. Presumably, the benzene ring of 4-aminophenol is subjected to two hydroxylation steps to yield 1,2,4-trihydroxybenzene. The proposed pathway differs from previously reported metabolic pathways for aniline and anilines with a methyl-, chloro-, sulfo-, or carboxyfunctional-group substituent at the C-4 position

Table 3 Identified intermediates during hydroquinone-degrading pathway of paracetamol using GC-MS and IC analysis

Intermediates	Structures	Intermediates	Structures
Paracetamol (acetaminophen)		4-aminophenol	
Hydroquinone		2-hexenoic acid	
Malonic acid		Oxalic acid	
Succinic acid		Formic acid	
Nitrate	NO_3^-	Nitrite	NO_2^-

(Fig. 5b) (Zhao et al. 2000; Hughes et al. 2002). The initial reaction in the degradation of anilines is catalyzed by a dioxygenase and yields the corresponding 1-amino-2-hydrodiols as the first metabolites. Subsequent oxidation of 1-amino-2-hydrodiols leads to the formation of the catechols. The dioxygenation and dehydrogenation steps to form catechols in these species are similar irrespective of which functional group at the C-4 position of aniline is the electron donor or electron acceptor. *P. putida* strain TW3 utilized 4-hydroxylaminobenzoate lyase-oxidized 4-hydroxylaminobenzoate to protocatechuate, replacing the amino group by a hydroxyl group (Hughes et al. 2002). Following the aromatic compounds, some carboxylic acids were detected by gas chromatography–mass spectrometry (GC-MS) and immediate constituent (IC) analysis, suggesting that the aromatic compounds can be the precursors of the carboxylic acids. To our knowledge, this is the first description of the biodegradation pathway for paracetamol. The initial steps are partially different from the reactions previously described for photocatalytic and electro-Fenton degradation of paracetamol (Brillas et al. 2009; Yang et al. 2009) (Fig. 5c), in which hydroquinone has been identified as the main transformation products. Gusseme et al. (2011) also reported that hydroquinone was a temporary transformation product of paracetamol degradation by two bacterial strains, *D. tsuruhatensis* and *P. aeruginosa*.

Hydroquinone pathway is the major route in paracetamol biodegradation. The metabolites of the initial steps in the paracetamol catabolic pathway were conclusively identified as 4-aminophenol and hydroquinone by GC-MS and HPLC-MS (Zhang et al. 2012). The main metabolic intermediates and some carboxylic acids and inorganics were determined by GC-MS and IC analysis, as listed in Table 3. During biodegradation of paracetamol (MSM, initial concentration 500 mg/L, 28 °C, 2 h, pH 7.2), a total of ten intermediates were identified. According to base structures of individual molecules, identified intermediates are categorized into four classes: (1) three aromatic compounds; (2) carboxylic acids, including five saturated and one unsaturated carboxylic acid; (3) nitrogen-containing straight chain compounds, including oxamic acid and acetamide; and (4) inorganics (ammonium and nitrate). Based on concentration profiles of identified intermediates during initial reaction stages (within 100 min), paracetamol mainly undergoes hydroxylation through OH addition onto the aromatic ring at para position with respect to the hydroxyl (–OH) group of paracetamol, as pathways shown in Fig. 2.

In contrast, in pyrocatechol pathway, as Wei et al. (2011) reported, acetyl group was replaced by hydrogen atom to form *p*-aminophenol as the first degradation product, and then *p*-aminophenol was further converted to the ring fission substrate *p*-phenol. Compounds 1 and 2 presented mass spectra with two major ions at *m/z* 108 and 109, which corresponded to a molecular mass $[M-H]^-$ of 4-aminophenol and

hydroquinone, respectively. Pyrocatechol and 2-aminoethanol were obtained by comparison of their mass spectrum with that of authentic derivatized compound. 4-Aminophenol was also identified as a main intermediate during photocatalytic degradation of paracetamol (Yang et al. 2009). Following formation of the three aromatic compounds, concentrations of carboxylic acids peaked around 270 min, suggesting that the three aromatic compounds can be precursors of the five carboxylic acids (Fig. 5) through cleavage of benzene rings and further biodegradation. While most intermediates exhibited a peak concentration, oxamic acid, ammonium, and nitrate continually increased in concentration throughout the reaction (up to 450 min), indicating continuous mineralization of nitrogen-containing intermediates. As suggested in pathway in Fig. 5c, oxamic acid can be formed through OH oxidation of acetamide after initial oxidation of paracetamol.

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