Consultant's Report

To

Integrated Laboratory Systems, Inc.

Use of *o*-Toluidine in the Manufacture of Dyes and on the Potential for Exposure to other Chemicals in the Processes involving *o*-Toluidine

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I. Abstract

This report pertains to the use of *o*-toluidine in the manufacture of dyes and the potential for exposure to other chemicals in the processes involving *o*-toluidine. The goal is to provide responses to a group of six specific questions involving *o*-toluidine as a precursor, intermediate, or product.

II. Introduction

The objective of this technical consultation is (i) to help characterize the likelihood and extent of human exposure to *o*-toluidine in a) *o*-toluidine manufacturing facilities and b) the aromatic amine dye manufacturing and use industry, and (ii) to help characterize the likelihood and extent of human co-exposure to other chemical agents that are suspect or known carcinogens or genotoxins (in human or non-human systems). The focus of this consultation is exposures experienced by workers in the historical cancer mortality and incidence cohort studies that have been conducted to date. Knowledge of the industrial processes, feedstocks, products and/or engineering and other controls will be required to evaluate the extent and level of exposure to *o*-toluidine and other co-exposures in these industries.

Exposure to *o*-toluidine also can occur in the rubber chemical manufacturing and use industry, and 4 major papers (Ward et al. 1991, 1996, Sorahan et al. 2000, Carreon et al. 2010) on epidemiology of cancer and/or exposure have been published, but were not provided by ILS, since these uses of *o*-toluidine do not involve the dye manufacturing industry. Background papers provided included one by Stasik 1988, which discusses the production and processing of 4-chloro-*o*-toluidine. This material has also been used as an intermediate in the manufacture of azo dyes.

III. Statement of Work

Responses to the following specific questions:

1. Is *o*-toluidine a necessary intermediate in the production of Magenta? Some of the epidemiologic studies on Magenta manufacture specifically mention use of *o*-toluidine (e.g., Rubino et al. for manufacture of Fuchsin or Safranine T), but others do not (e.g. Case and Pearson 1954), so we want to know if the presence of *o*-toluidine in those manufacturing plants can be inferred from knowledge of the synthetic process.

    - The short answer is "it depends"; but the full answer lies with understanding the structures involved. At least 4 structures are associated with the Magenta "family" of dyes (cf. Fig. 1). These dyes were originally produced in the 19th century, from the oxidation of commercial aniline. At that time, aniline contained significant amounts of *o*- and *p*-toluidines. Ironically, it is unlikely that any of these dyes would exist if pure aniline had been employed in the mid-19th century. With this perspective in mind, it is clear that Magenta 0 (Basic Red 9; CI 42500) does not require *o*-toluidine. Instead *p*-toluidine is needed, along with aniline itself, in a ratio of 1:2. However, Magenta I (Fuchsin, Basic Violet 14; CI 42510), Magenta II, and Magenta III (Basic Violet 2; CI 42520) do require *o*-toluidine, with aniline also needed for Magenta I, as illustrated in Fig. 2. Here, it can be seen that the methyl (-CH_3) group of *p*-toluidine becomes the central C-atom in the Magenta dye structure. The mention of aniline but not *o*-toluidine in the Case and Pearson paper recognizes, without expressly stating it, the presence of toluidines (*o*/*p*) as aniline impurities.

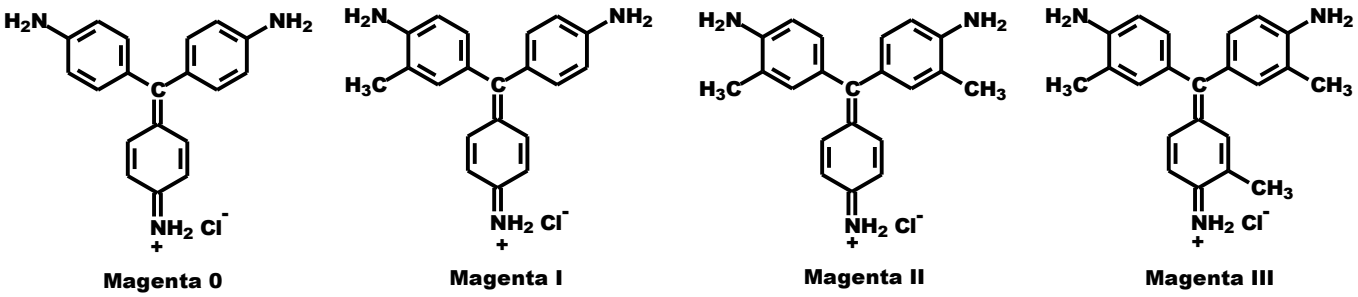


Fig. 1. Structures of Magenta dyes, each derived from oxidation of impure aniline.

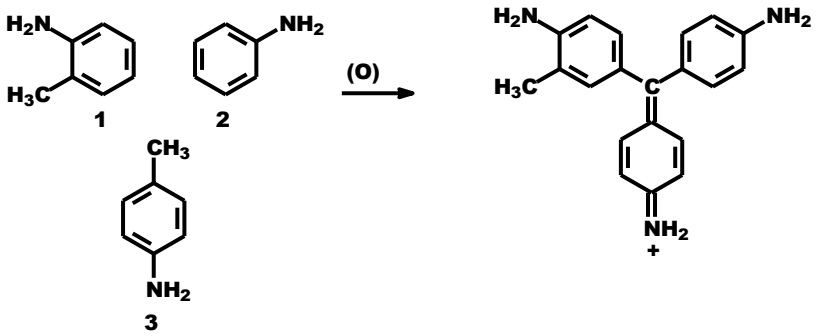


Fig. 2. Magenta I dye formation from a mixture of *o*-toluidine (**1**), aniline (**2**), and *p*-toluidine (**3**).

- Similarly, Mauveine, the very first commercial synthetic dye, and Safranine T (cf. Fig. 3) were obtained from "crude" aniline containing *o*- and *p*-toluidine as impurities.

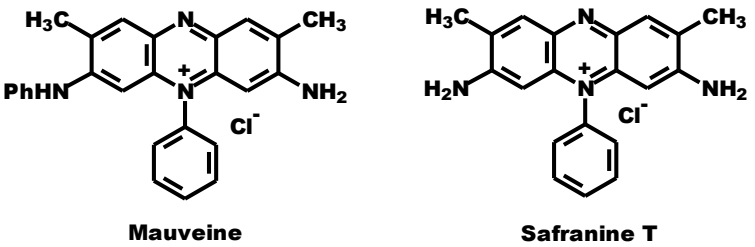


Fig. 3. Structures of Mauveine and Safranine T (Basic Red 2, CI 50240).

Cohort-specific discussion of exposures to o-toluidine from manufacture of magenta and other molecules

a. Case and Pearson cohort:

(1) There are 2 exposure groups of possible concern – the aniline group and the magenta group (Table 1, p. 214, Case and Pearson 1954).

- Is it reasonable to assume that the first aniline manufacturing group (n = 812) were also exposed to o-toluidine as an impurity? If so, as a contaminant or an intermediate or both?

This seems reasonable; but I do not know when aniline was first available, commercially, in pure form. Due to the higher cost of the pure form, it is likely that technical grade aniline, which contained *o*-toluidine as a contaminant, was still used.

- What if any other chemicals might they have been exposed to? P-toluidine? Others?

The key to following the possibilities lies with the chemistry involved in aniline formation (cf. Fig. 4 below). Aniline is produced in two steps from benzene, viz. nitration and reduction. The presence of toluene as a petroleum-based contaminant in benzene leads to a mixture of toluidines (*o*, *m*, *p*) from the same 2 steps. The lower sequence disappears when pure benzene is used. Note also, that very small amounts of *m*-toluidine would form, owing to the low level of its precursor, *m*-nitrotoluene.

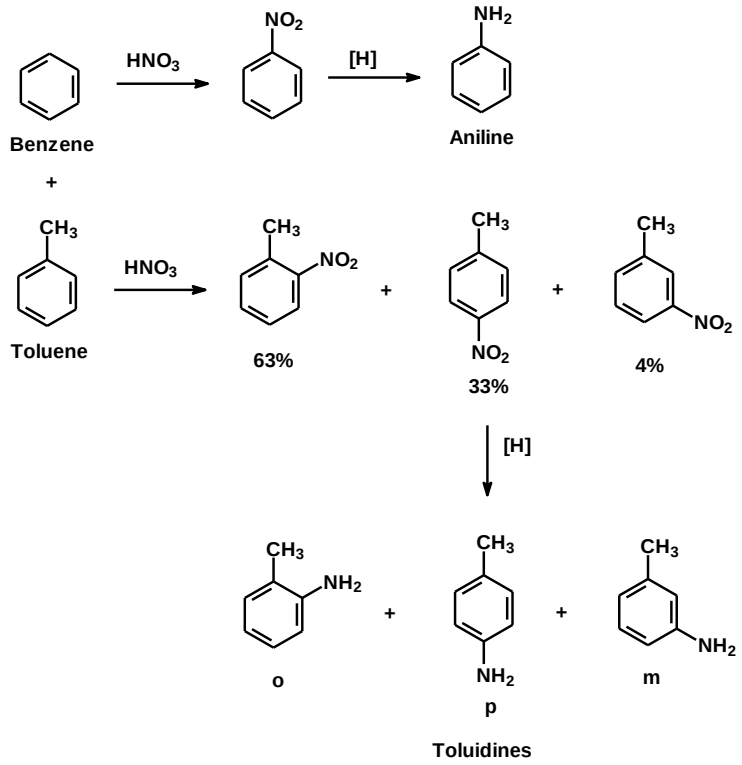


Fig. 4. Chemistry leading to simultaneous aniline and toluidine (*o*, *m*, *p*) formation.

- Would exposure to o-toluidine and other agents have changed significantly between the early 1900's and 1954? (Note that some members of this cohort may have had exposure as early as 1910 or so; see Case et al. 1954).

Probably YES, due to the availability of higher quality benzene and aniline by the 1950s. It is worthwhile to point out that high grade solvents were more important in the pharmaceutical industry than in textile dye manufacturing. In the latter case, the color delivered to a substrate rather than homogeneity was more critical.

(2) Based on the fact that Magenta I, II and III appear to require o-toluidine-

- According to the report, is it reasonable to assume that the magenta manufacturing group (n = 85; Table 1, p 214) would have been exposed to o-toluidine?

YES; there was no other way to have formed the dye.

- (It does not appear that we can tell what types of magenta were produced, but were they being made at that time?)

One should expect Magenta I to be dominant because it reflects an o-toluidine composition more consistent with its presence as a contaminant in aniline. Note that Magenta II and III do not require aniline at all.

- Would they also be exposed to aniline and p-toluidine?

YES; there was no other way to have formed Magenta I.

- What, if any, other chemicals might they have been exposed to? Presumably, given the date of this study, there would have been no 4'4-methylene bis (2-methyaniline) involved.

If the plant made its own aniline, then the door opens to exposures to nitroaromatics shown in Fig. 1 above.

(3) Are any of the other exposure groups reported in Table 1 likely to be of concern with respect to o-toluidine exposure?

No others are apparent.

b. Rubino et al. (1982) cohort:

The authors state that the manufacture of "new fuchsin" ("new magenta") and safranine T involves o-toluidine and the following:

The full processes for obtaining fuchsin and safranine were carried out in two separate departments located in two different buildings within the area of the factory. In the first department (which we will call Section I), synthesis of o-toluidine and 4,4'-methylene bis(2-methylaniline) was carried out according to the sequence illustrated in Fig. 2. In the second department (Section II), processes for obtaining fuchsin and safranine T were carried out. A mixture of o-toluidine, 4,4'-methylene bis(2-methylaniline), and o- nitrotoluene was heated to obtain fuchsin. The operation included the recovery of excess o-toluidine at the end of the process. Safranine T was obtained by oxidizing a mixture of o-toluidine and 2,5-diaminotoluene in the presence of aniline. o- Aminoazotoluene was the intermediate of the reaction.

- Do we know whether exposure to o-toluidine was likely to be predominant and what might be the relative levels of these specified co-exposures compared with each other and with exposure to o-toluidine?

o-Toluidine is definitely the common thread, in that it is required for Safranine T, Fuchsin, and 4,4'-methylene bis(2-methylaniline) formation.

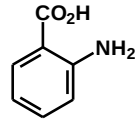
c. Ott and Langner 1983 cohort:

(1) The authors indicate that o-toluidine would only be used in the manufacture of thioindigo, not bromoindigo or indigo itself (Table 1, p 764).

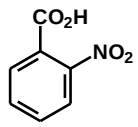
- First, do we know whether the use of/exposure to o-toluidine in thioindigo manufacture was likely to be predominant and what might be the relative levels of the specified co-exposures (1,2 dihydroacenaphthylamine, 4-chloro-o-toluidine, thiooxyl, 2-amino benzoic acid) compared to each other and to o-toluidine?

(2) Is there any reason to suppose that o-toluidine might in fact be used in the manufacture of bromoindigo or indigo manufacture?

The possible use of *o*-toluidine in thioindigo formation is surprising to me. I am aware of sequences employing anthranilic acid and *o*-nitrobenzoic acid to give the indigoid and thioindigoid systems but not *o*-toluidine. The latter route would be an unconventional method.



Anthranilic acid



o-Nitrobenzoic acid

d. Stasik 1988 cohort:

Of the 3 other chemicals mentioned in 4-chloro-o-toluidine manufacture (N-acetyl-o-toluidine, 6-chloro-o-toluidine, o-toluidine), what would be the likely relative levels of use of/exposure to these chemicals compared to each other and to exposure to 4-chloro-o-toluidine?

Based on the chemistry associated with this set of interrelated compounds (cf. Fig. 5), it is likely that exposures to *N*-acetyl-*o*-toluidine, 4-chloro-*o*-toluidine, *o*-toluidine would be comparable, if the intermediate compounds (*N*-acetyl-*o*-toluidine and chloro- *N*-acetyl-*o*-toluidines) were isolated prior to the next step. As the reaction scheme shows, 4-chloro-*o*-toluidine flows from the acetylation of *o*-toluidine to give *N*-acetyl-*o*-toluidine, followed by chlorination and hydrolysis to give 4-chloro-*o*-toluidine. Note also that 6-chloro-*o*-toluidine arises from simultaneous chlorination in the 6-position of *N*-acetyl-*o*-toluidine and that this product carries over to the hydrolysis step, giving 6-chloro-o-toluidine as a by-product.

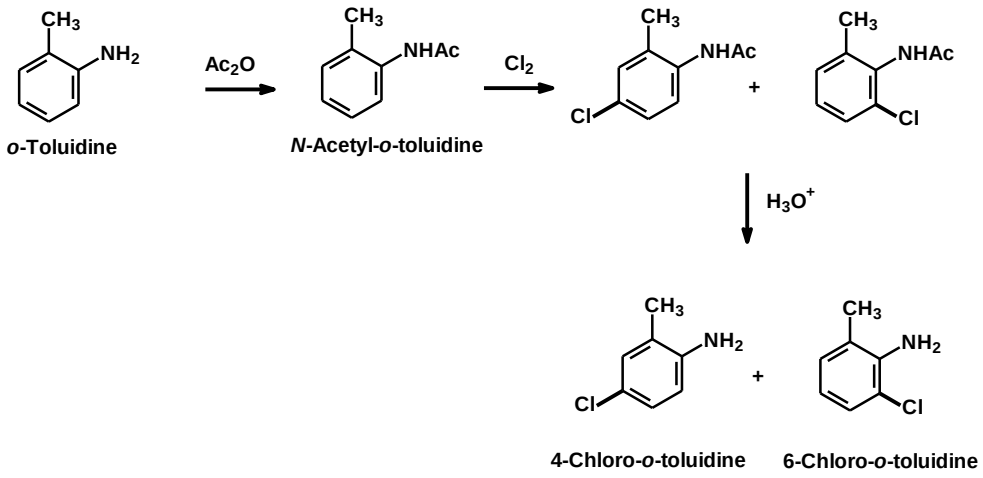


Fig. 5. Chemistry associated with the 4/6-chloro-o-toluidines.

2. With respect to *o*-toluidine exposure in cohorts where there are no quantitative or semi-quantitative data on exposure to *o*-toluidine, can either ambient or dermal exposure be inferred from knowledge of the industrial process as a precursor, intermediate, end or side product? If so, among which workers?

   - Clearly, the types of possible exposures would depend on whether protective measures were employed. Since the adoption of OSHA regulations in the mid-1970s, lab coats and gloves have been used, to guard against dermal exposures. Where these regulations were followed, dermal exposures would not be an issue. In addition, standards pertaining to air-flow (ventilation) in the work place were adopted, to control air-borne exposures. Most domestic plants provided disposable air masks to their workers as well.
   - Prior to the adoption of formal regulations, both types of exposures from industrial processes involving *o*-toluidine usage can be inferred, especially among workers responsible for manually transferring this chemical to reaction vessels for dye manufacturing.

3. What is the historical vs. more recent likelihood of exposure, and changes in exposure over time, taking into account the overall time span of the cohort studies under consideration?

   - In 1994, the newly adopted German Consumer Goods Ordinance restricted the use of certain azo dyes in consumer goods that often came in contact with the skin. The policy indicated that no consumer goods could be marketed in Germany that contained azo dyes derived from any of the group of 22 aromatic amines designated as cancer suspect agents. These goods included clothing, bedding, towels, hairpieces, wigs, hats, various sanitary items, sleeping bags, footwear, gloves, wristwatch straps, handbags, purses/wallets, briefcases, chair covers, purses worn round the neck, textile or leather toys and toys which included textile or leather garments, yarn and fabrics intended for use by the final consumer. *o*-Toluidine and 4-chloro-*o*-toluidine were among the restricted aromatic amines.
   - The concern was that these dyes could enter the human body and undergo metabolism (reductive-cleavage of the azo group) to release aromatic amines used in their manufacture (cf. Fig. 6). In the present example, *o*-toluidine would be released.

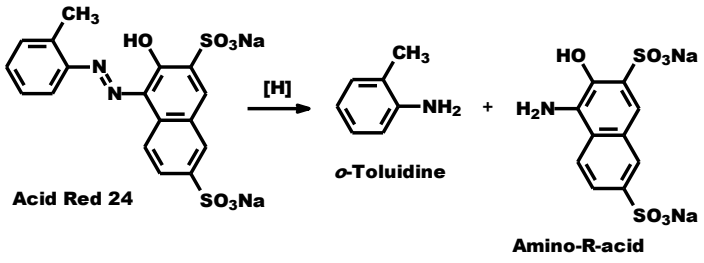


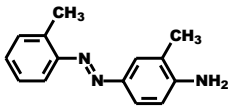
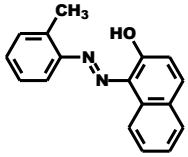
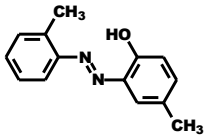
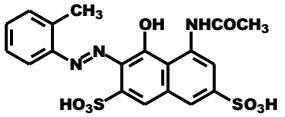
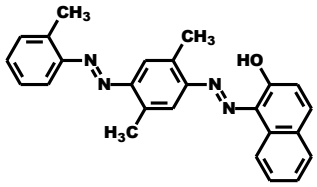
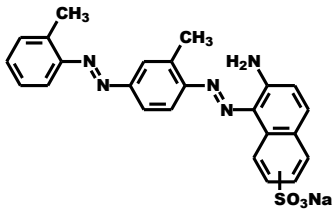
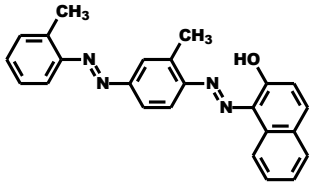
Fig. 6. Release of *o*-toluidine via metabolic reductive cleavage of an azo dye.

   - Prior to an awareness of toxicological concerns regarding *o*-toluidine, azo dyes such as the 30 listed below were manufactured and registered in the Colour Index. Since then, only those 10 dyes in bold are still listed as having commercial products.

o-Toluidine Based Dyes	Colour Index Designations
Solvent Yellow 3	**11160**
Solvent Yellow 6	11390
Solvent Yellow 12	**11860**
Solvent Red 2	**12005**
Solvent Orange 2	**12100**
Pigment Chrome Yellow L	12720
New Yellow RMF	13130
Cochineal Scarlet 2R; Helio Red BL	14810
Acid Orange 16	**16011**
Acid Red 25:1	16047
Acid Red 24	16140
Direct Red 65	17870
Acid Red 35	**18065**
Acid Red 265	**18129**
Direct Red 142	19500
Diazo Brilliant Scarlet B	19565
Direct Red 119	19590
Naphthamine Fast Bordeaux BR	19610
Solvent Orange 13	26075
Solvent Red 24	**26105**
Solvent Red 26	**26120**
Acid Red 104	**26420**
Cloth Red 2B	26430
Acid Red 148	26665
Azo Cerise M	26765
Croceine 3B	26785
Cloth Scarlet R	26910
Acid Red 177	27015
Acid Red 115	27200
Direct Violet 5	27660

- Among the current *o*-toluidine derived dyes, the Colour Index indicates that Acid Red 35 comprises 18 commercial products, with 81 for Solvent Red 24, 21 for Solvent Red 26, 12 for Solvent Orange 2 8 for Solvent Yellow 3, 7 for Solvent Yellow 12, 5 for Solvent Red 2, 4 for Acid Orange 16, 2 for Acid Red 265, and 2 for Acid Red 104. Structures associated with these dyes are illustrated in the following table.

- **Table 1. Representative *o*-toluidine based commercial dyes and pigments**

Colour Index name	Chemical Structure
Solvent Yellow 3	
Solvent Orange 2	
Solvent Yellow 12	
Acid Red 35	
Solvent Red 26	
Acid Red 104	
Solvent Red 24	

- It should be added that a substantial majority of present-day manufacturing involving these dyes seems to occur outside the USA and Europe, in plants in India and China.

4. What other potentially carcinogenic exposures can be inferred from the industrial process, either as a precursor, intermediate, or product, whether or not mentioned by authors, a) in areas where *o*-toluidine is used and/or b in areas/job where workers who are potentially exposed to *o*-toluidine might have some contact with them?

- Two examples are 4-chloro-*o*-toluidine and 4-amino-2',3-dimethylazobenzene, both of which are co-listed with *o*-toluidine among the group of 22 aromatic amines in the German Consumer Goods Ordinance. Both are also manufactured from *o*-toluidine, as illustrated in Fig. 7. Similarly, 4-chloro-*o*-toluidine has been used to make dyes such as Disperse Red 220.
- Note also from the previous table that Acid Red 104 and Solvent Red 24 are derived from the carcinogen 4-amino-2',3-dimethylazobenzene, making the associated manufacturing a potential exposure to this carcinogen and *o*-toluidine.

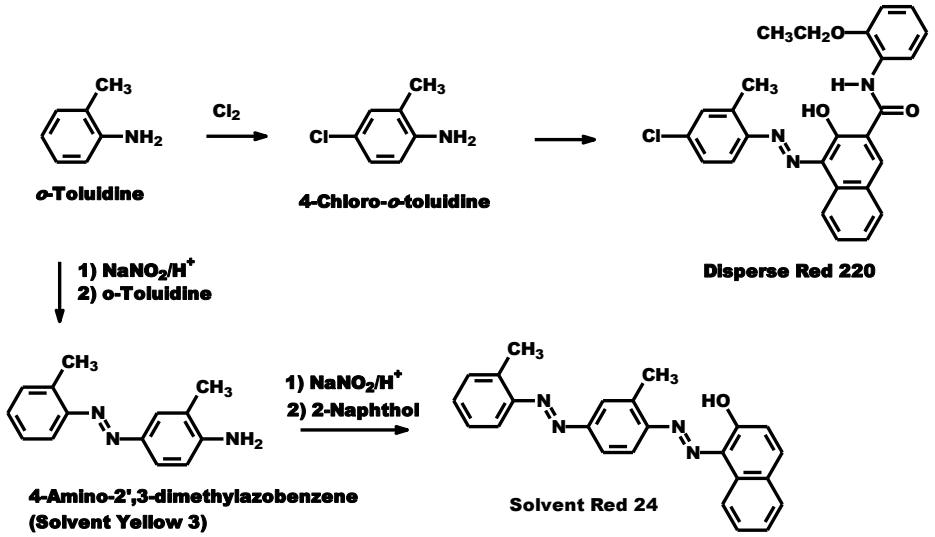


Fig. 7. Examples of *o*-toluidine based manufacturing that involve other potential carcinogenic exposures.

- Although organic pigments were not included in the German Goods Ordinance, due to the insolubility of these compounds, it is worthwhile pointing out that several current commercial pigments are derived from *o*-toluidine. They include Pigment Red 95, Pigment Red 148, and Pigment Red 253, all of which require the product obtained from reacting *o*-toluidine with beta-oxynaphthoic acid (cf. Fig. 8).

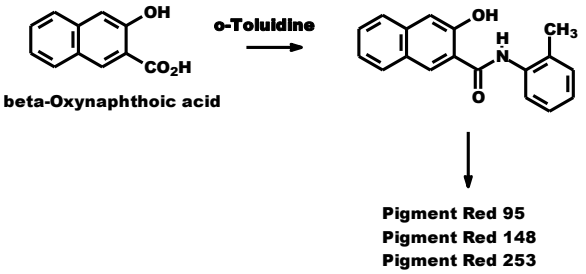
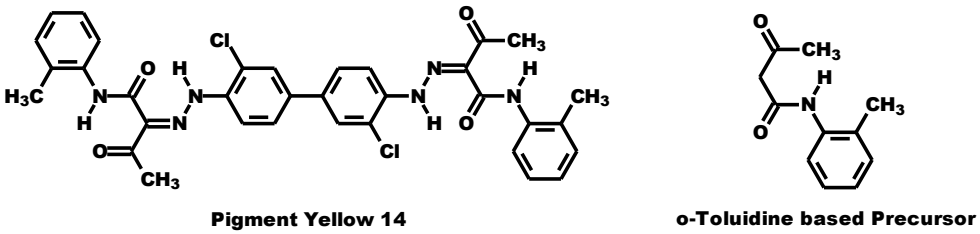


Fig. 8. Organic pigment formation employing *o*-toluidine.

- Further, the widely used organic colorant Pigment Yellow 14, with its 193 commercial listings in the Colour Index is an *o*-toluidine based compound. Manufacturing of this colorant also requires 3,3'-dichlorobenzidine, another aromatic amine associated with the German Goods Ordinance. In this case, it is possible that pigment manufacturing does not involve exposure to both *o*-toluidine and 3,3'-dichlorobenzidine. Here, the precursor involving *o*-toluidine is probably made elsewhere and purchased for coupling with 3,3'-dichlorobenzidine.



5. If any such co-exposures are identified, what could be the likelihood of a) ambient and b) dermal exposure by workers engaged in the process? (e.g., would the substance only be used in a closed process?) and what is the likely level of exposure relative to that of *o*-toluidine itself (e.g., is the co-exposure the predominant or minor exposure relative to that of *o*-toluidine?)

- With 81 products listed in the Colour Index, by far the most important of the o-toluidine based dyes is Solvent Red 24. This dye is made in two steps from *o*-toluidine, with the carcinogen Solvent Yellow 3 serving as an essential intermediate (Fig. 5). Key exposures would involve *o*-toluidine, as the monoazo dye intermediate is normally generated and used *in situ*. Solvent Red 24 is used as a colorant for hydrocarbon solvents, oils, waxes, and petroleum, rather than for traditional consumer goods. Due to the low volatility of the final and precursor dyes, ambient exposure would be less likely than dermal exposures.

6. What is the historical vs more recent likelihood of exposure to these co-exposures, and changes in exposure over time, taking into account the overall time span of the cohort studies under consideration?

- With far fewer dyes currently in the marketplace that are derived from *o*-toluidine and 4-chloro-*o*-toluidine, these co-exposures have likely decreased. In addition, the great majority of current exposures take place outside the USA.

- The potential for enhanced dermal penetration from dye–solvent mixtures has not been reported. This could be an issue for carcinogenic solvent dyes.

IV. Report Summary

o-Toluidine (**1**) has been a dye precursor since the mid-19th century when the first synthetic dye (Mauveine) was produced as an act of serendipity. In the 100 years that followed, this aromatic amine functioned as a precursor for a significant number of dyes and pigments used in the coloration of a wide variety of consumer goods. Following the recognition of its carcinogenicity in laboratory animals and its potential for causing cancer in humans, *o*-toluidine manufacture and its use in dye manufacturing has been largely banned in the western world and in many parts of the east, and the number of dyes based on this compound has dropped dramatically. An overview of the structures of historical and current dyes derived from *o*-toluidine is presented.

Nowadays, *o*-toluidine is used mainly in the manufacturing of solvent dyes (e.g. Solvent Red 24) for petroleum products and organic pigments (e.g. Pigment Yellow 14) for paints and plastics, with both types of colorants still regarded as important. *o*-Toluidine is also essential in the production of 4-chloro-*o*-toluidine and 4-amino-2',3-dimethylazobenzene, both of which are also used in dye manufacturing. This has opened the door to potential co-exposures to carcinogenic compounds.

Present-day safety regulations help limit dermal and ambient exposures to *o*-toluidine, in cases where this compound is still used. This is important since most but not all historical exposures have been eliminated on both sides of the globe.