The influence of airborne N,N-dimethylformamide on liver toxicity measured in industry workers: A systematic review and meta-analysis

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Abstract
Background: Modern industry is developing and so is the consumption of N,N-dimethylformamide (DMF) and the occupational population exposed to DMF. However, chronic occupational and experimental exposure to DMF has been especially linked to liver and gastrointestinal disturbances. Aims: This study aims to systematically review and evaluate with a meta-analysis the influence of DMF exposure on human liver toxicity. Methods: The PubMed/Medline, the ECHA restriction dossier and the Web of Science were searched. Midpoint DMF exposure levels were calculated, and the association between DMF exposure and liver toxicity was investigated. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Results: Of 92 screened articles, 19 articles were included in the review and of them, 10 articles were included in the meta-analysis. No association was observed when the midpoint DMF exposure was less than 20 mg/m3 (OR: 1.58, 95% CIs: 0.68–3.65). A positive association between DMF exposure and liver toxicity was observed when the midpoint DMF exposure was between 21 mg/m3 and 25 mg/m3 (OR: 3.26, 95% CIs: 1.38–7.73). Conclusions: Higher exposure DMF levels are associated with liver toxicity. However, these results tend to overestimate potential risks because the use of midpoint exposures includes and gives weight to populations at the upper end of the exposure distributions and because liver toxicity was defined as a statistical significant difference in liver enzyme levels compared to control groups, which is not identical to biologically relevant effects and adverse health effects.

Keywords
Liver toxicity, N, N-dimethylformamide, risk assessment, epidemiology, systematic review

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Introduction
N,N-dimethylformamide (DMF; CAS 68-12-2) is a universal aprotic organic solvent widely used in occupational settings, including synthetic leather, plastic industries and pharmaceutical companies1 and also within the man-made fibre industry and the PU coatings and membranes industry.2 As modern industry is developing so is the consumption of DMF and the occupational population exposed to DMF.3 DMF can pass through the skin and can pose, together with its inhalation, health risks to various organs.

A human volunteer study reported that about 40% and 60% of the total DMF uptake was contributed by skin and...
inhalation routes, respectively. Another field study found that, for the same DMF-exposed manufacturing workers, skin exposure from liquid contact as well as skin vapor could account for 60% of the human internal exposure dose in a low DMF exposure setting (DMF < 2 ppm), predominantly over that from inhalation exposure of 15% during high-exposure task. Dermal exposure to DMF over five consecutive days can result in the accumulation of a significant body burden. For substances like DMF which are readily absorbed through the skin, total exposure by inhalation and dermal exposure may be biomonitored by urinary NMF, a parameter used by industry over many years. The metabolism of DMF begins with its oxidation to N-hydroxy-N-methylformamide (HMMF) by cytochrome P2E1 (CYP2E1). HMMF further breaks down to form N-methylformamide (NMF), which in turn is oxidized to a reactive intermediate most likely methyl isocyanate. NMF in urine has been, therefore, used to evaluate DMF exposure in occupational settings.

Chronic occupational and experimental exposure to DMF has been linked especially to liver and gastrointestinal disturbances. As DMF is primarily metabolized in the liver, the liver cell is the first target of the toxic actions of DMF exposure. Impaired cells release enzymes, such as gamma-glutamyltransferase (γ-GT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), whose abnormal values are indicative of liver damage.

Elevated serum-level enzymes can reflect abnormalities in liver cells or in the bile duct. Predominant elevation of aminotransferases (ALT and AST) typically indicates hepatocellular injury, and elevated gamma-glutamyltransferase (γ-GT) suggests cholestatic liver injury. Both AST and ALT are released into the blood in greater amount when hepatocytes are damaged. γ-GT elevation is the most sensitive marker of hepatobiliary disease, although it cannot by itself indicate a specific cause of liver toxicity.

Several independent scientific committees have assessed the toxicological profile of DMF confirming this hypothesis. In addition, it has been suggested that for Asian, especially Chinese populations, specific genetic polymorphisms may be associated with DMF-induced abnormal liver function.

Occupational exposure limits (OELs) for DMF of 15, 18–20 20 mg/m³ have been proposed, which are based primarily on liver toxicity in experimental animals and exposed workers. Short-term peak exposures by a factor of 2 have been defined as short-term exposure limits.

For derivation of an OEL based on liver toxicity, either animal data or human information may be used. The latter should be preferred because there are no uncertainties regarding interspecies extrapolation. Reliable information for DMF exposure has been obtained during the last decades for a total of more than 300 exposed European and 800 Asian workers. A systematic review of this information offers new insights that can be useful when defining an OEL or derived no-effect level.

To the best of our knowledge, this is the first study to systematically review the available human literature and which evaluates via a meta-analysis the influence of DMF exposure on liver toxicity.

**Methods**

**Search strategy**

The PubMed/Medline, the ECHA restriction dossier and the Web of Science databases were systematically searched to retrieve relevant articles published up to June 2019. The searched string used in PubMed/Medline was (((((DMF* OR DMF*[Title/Abstract])) AND ((liver* OR (hepat* OR liver*[Title/Abstract])) AND (((((AST OR ALT OR γ-GT) AND (liver toxicity OR liver function OR hepatotoxicity))) AND (human*)))). The search was further restricted in articles written in English and referred to humans. The search string used in the Web of Science database was (((((DMF* OR DMF*[Title/Abstract]))) AND ((liver* OR (hepat* OR liver toxicity OR liver function OR hepatotoxicity))) AND (human))). The search was further restricted in articles written in English. For the articles retrieved by the ECHA dossier, there was no search string used as the articles included in the dossier were all relevant and specific to DMF and its effect on liver toxicity.

In addition, we investigated the references of the retrieved articles related to the topic to make sure no article was missed.

**Study selection**

The first author (EA) searched for articles in the databases. EA screened the titles and abstracts and evaluated the articles retrieved via the databases. The articles that met the eligibility criteria were screened and full texts were read by EA. The second author (HPG) searched for and read the full-text articles in the ECHA restriction dossier. The reason for the exclusion of any article excluded after full-text screening is reported.

**Eligibility criteria**

The inclusion criteria were (1) human studies, (2) studies including factory workers exposed to DMF with no other systematic disease, (3) studies with values of the following serum hepatic enzymes (AST, ALT and γ-GT) and (4) for the meta-analysis studies having the sufficient data to calculate the odds ratios (ORs) and 95% confidence intervals (CIs). Participants with at least one of the three enzyme activity levels significantly greater than the levels of the control group were considered as cases with liver damage.

The exclusion criteria were (1) animal studies, case reports, conference papers and abstracts; (2) studies with insufficient quantitative exposure data; (3) studies without concurrent control groups (these are excluded only from...
the meta-analysis); and (4) studies not reported in English language.

**Data extraction**

The first authors’ name, year of publication, number of workers exposed to DMF and number of healthy controls, means, medians, minimum and maximum values of DMF exposure and midpoint value of DMF exposure were extracted from the articles.

**Qualitative assessment**

The Newcastle–Ottawa scale (NOS) was applied for the quality assessment of articles with a maximum score of nine.22 The quality of each study was considered high if score ≥ 7 was obtained. Quality evaluation was done by two of the authors (EA and MPZ). Each study was judged on three broad perspectives: the selection of the study groups, the comparability of the groups and the ascertainment of the exposure of interest.

**Meta-analysis**

In order to investigate publication bias, funnel plots were constructed plotting the logarithmically transformed ORs against the standard error (SE) of the associated log (OR). The distribution of study risk estimates across the funnel plot was examined visually and Egger’s test23 for small study effects was performed to assess the degree of asymmetry. Midpoint DMF exposure values were calculated based on the reported low and high DMF exposure values.

The following categories of DMF exposure were constructed: (1) DMF exposure ≤ 20 mg/m³, (2) DMF exposure ≥ 21 mg/m³ and DMF exposure ≤ 25 mg/m³ and (3) DMF exposure ≥ 26 mg/m³.

Two-way contingency tables based on exposed to DMF and non-exposed to workers and their liver toxicity status were constructed. Logarithmic ORs and SEs were calculated using the method of Woolf.24 The association between DMF exposure and liver toxicity is expressed in ORs with the accompanying CIs.

A random effects model was applied in all meta-analyses procedures to account for potential variation (heterogeneity) in study outcome between studies. In a random effect model, the inverse variance of the effect estimates within each study added with a constant to represent the between study variance is used as weight factors. The $I^2$ (I-squared statistic) was used to describe the percentage of variation across studies due to heterogeneity.25

The association between DMF exposure and liver toxicity in exposed compared to non-exposed workers was investigated firstly, in a dose–response subgroup analysis, and secondly, in an overall analysis where the results of studies with different dosages were pooled together.

In addition, a leave-out-one-study sensitivity analysis was done after excluding each study to assess the stability of the results. If it did not significantly change the estimation of the pooled data, the stability or consistency of the analyses would be confirmed. Moreover, due to differences in the metabolism of DMF between Europeans and Asians, a subgroup analysis only with European studies was performed.

**Results**

**Study selection**

Two hundred and twenty-one articles were retrieved from the databases. After removing the duplicates (n = 129), 92 articles remained and screened (see flow chart). Of those, 60 articles were excluded based on their title/abstract because they were not related to the subject. Full-text copies of 32 articles were assessed for eligibility, of which 13 articles were excluded as non-relevant to the topic. Finally, 19 studies were included in this review.

To be included in the subsequent meta-analyses, studies had to fulfil additional criteria or would be otherwise excluded. One study did not state the level of DMF concentration10 and another study26 was excluded as it only provided information on cumulative exposure to DMF, and as such it was not possible to harmonize this statistical information with the rest of the studies. The study by Lauverys et al.27 was excluded from the meta-analysis as the exact number of the exposed and non-exposed workers with liver toxicity was not reported. For the purposes of the specific meta-analyses, studies that did not include a control (non-exposed) group28–31 were excluded from the analysis. The study by Major et al.32 was excluded because of not providing sufficient information on liver toxicity. Further information may, however, have been reported in a publication that was not written in English (see flow chart). Finally, 10 studies were analysed in the meta-analysis (Table 1).

**Study characteristics**

The basic features of all the articles in the review and the meta-analysis can be found in Table 1. The studies included were published between 1980 and 2019. The number of workers with liver toxicity and the number of healthy controls is presented alongside the minimum and maximum value of the DMF exposure, the mean, medians and the estimate midpoint value of the DMF exposure. The studies that were used in the review but were not eligible to be included in the meta-analysis are presented in Table 1 as well.

The total score of quality of all articles included in this meta-analysis is shown in Table 2. Most studies met or exceeded the NOS criteria and therefore we can be assured that the studies included in the meta-analysis are of
Table 1. Study characteristics of the articles used in the qualitative review and the meta-analysis.

| First author, publication year | Total N | N\(^b\) toxicity | N, no toxicity | Min DMF exposure (mg/m\(^3\)) | Max DMF exposure (mg/m\(^3\)) | Mean DMF exposure (mg/m\(^3\)) | Median DMF exposure (mg/m\(^3\)) | Estimated midpoint DMF value\(^c\) exposure (mg/m\(^3\)) |
|-------------------------------|---------|------------------|----------------|-----------------------------|-------------------------------|-------------------------------|----------------------------------|---------------------------------|
| Cirla et al.\(^{33}\) Exposed | 100     | 25               | 75             | 8                           | 58                            | 22                            | Not reported                     | 33                              |
| Non-exposed                  | 100     | 10               | 90             |                             |                               |                               |                                  |                                 |
| Wang et al.\(^{34}\) Exposed | 80      | 6                | 74             | 30                          | 180                           | Not reported                   | Not reported                     | 105                             |
| Non-exposed                  | 54      | 2                | 52             |                             |                               |                               |                                  |                                 |
| Cai et al.\(^{35}\) Exposed  | 111\(^d\) | 4               | 22             | 3                           | 27                            | 13.5                           | Not reported                     | 15                              |
| Non-exposed                  | 142\(^d\) | 2               | 9              |                             |                               |                               |                                  |                                 |
| Fiorito et al.\(^{36}\) Exposed | 75     | 17               | 58             | 2                           | 40                            | Not reported                   | Not reported                     | 21                              |
| Non-exposed                  | 75      | 3                | 72             |                             |                               |                               |                                  |                                 |
| Wrbitzky\(^{37}\) Exposed    | 126     | 22               | 104            | 0.3                         | 300                           | 12.3                           | 3.6                              | 150.1                           |
| Non-exposed                  | 54      | 7                | 47             |                             |                               |                               |                                  |                                 |
| He et al.\(^{38}\) Exposed   | 46      | 9                | 37             | 2.5                         | 36                            | Not reported                   | Not reported                     | 19.3                            |
| Non-exposed                  | 33      | 3                | 30             |                             |                               |                               |                                  |                                 |
| He et al.\(^{39}\) Exposed   | 72      | 21               | 51             | 9.8                         | 36.2                           | 18.6                           | Not reported                     | 23                              |
| Non-exposed                  | 72      | 3                | 69             |                             |                               |                               |                                  |                                 |
| Kilo et al.\(^{40}\) Exposed | 200     | 30               | 170            | 0.1                         | 46.9                           | 6.1                            | 3.13                             | 23.5                            |
| Non-exposed                  | 174     | 20               | 154            |                             |                               |                               |                                  |                                 |
| Wu et al.\(^{41}\) Exposed   | 698     | 64               | 634            | 12.7                        | 30                            | Not reported                   | Not reported                     | 21.4                            |
| Non-exposed                  | 188     | 8                | 180            |                             |                               |                               |                                  |                                 |
| Catenacci et al.\(^{42}\)   | 54      | 1                | 6              | 1.8                         | 25                            | Not reported                   | 13.4                            |
| (matched pairs)\(^a\) Controls toxicity | 54     | 4                | 43             |                             |                               |                               |                                  |                                 |
| (matched pairs)\(^a\) Controls no toxicity | 54     | 4                | 43             |                             |                               |                               |                                  |                                 |

Total N: N, Cases toxicity  N, Cases no toxicity

(continued)
Table 1. (continued)

| First author, publication year | Total N | N\textsuperscript{b} toxicity | N, no toxicity | Min DMF exposure (mg/m\textsuperscript{3}) | Max DMF exposure (mg/m\textsuperscript{3}) | Mean DMF exposure (mg/m\textsuperscript{3}) | Median DMF exposure (mg/m\textsuperscript{3}) | Estimated midpoint DMF value\textsuperscript{c} exposure (mg/m\textsuperscript{3}) |
|--------------------------------|---------|-------------------------------|---------------|------------------------------------------|------------------------------------------|--------------------------------------------|---------------------------------------------|-----------------------------------------------|
| Lauwerys et al.\textsuperscript{27} | Exposed | 22                            | Not reported  | 1                                        | 46.6                                     | 23.0                                       | 23.8                                        |                                 |
|                                 | Non-exposed | 28                            | Not reported  | Not reported                             | Not reported                             | Not reported                               | Not applicable                              |                                 |
| Yonemoto and Suzuki\textsuperscript{29} | Exposed | 9                             | 0             | 1                                        | 15                                       | Not reported                               | Not reported                               | 8                              |
|                                 | Non-exposed | 0                             | 0             | 0                                        | Not reported                             | Not reported                               | Not applicable                              |                                 |
| Redlich et al.\textsuperscript{43} | Exposed | 46                            | 35            | Not reported                             | Not reported                             | Not reported                               | Not reported                               | Not applicable                          |
|                                 | Non-exposed | 0                             | 0             | 0                                        | Not reported                             | Not reported                               | Not applicable                              |                                 |
| Yang et al.\textsuperscript{28} | Exposed | 13                            | 3             | Not reported                             | Not reported                             | Not reported                               | Not applicable                              |                                 |
|                                 | Non-exposed | 0                             | 0             | 0                                        | Not reported                             | Not reported                               | Not applicable                              |                                 |
| Sakai et al.\textsuperscript{30} | Exposed | 10                            | 0             | 10                                       | 7.5                                      | 31.2                                       | Not reported                               | 19.35                                      |
|                                 | Non-exposed | 0                             | 0             | 0                                        | Not reported                             | Not reported                               | Not applicable                              |                                 |
| Major et al.\textsuperscript{32} | Exposed | 26                            | 6             | 20                                       | 0.6                                      | 24                                         | Not reported                               | 12.3                                       |
|                                 | Non-exposed | 32                            | 0             | 0                                        | Not reported                             | Not reported                               | Not applicable                              |                                 |
| Luo et al.\textsuperscript{44} | Exposed | 176                           | 45            | 131                                      | 0.3                                      | 260                                        | 34.8                                       | 130.1                                      |
|                                 | Non-exposed | 0                             | 0             | 0                                        | Not reported                             | Not reported                               | Not applicable                              |                                 |
| Qi et al.\textsuperscript{26} | Exposed | 429                           | 41            | 388                                      | Cumulative dose (mg/m\textsuperscript{3}) × year (>0–6.6) | 7.0                                         | Not reported                               | Not applicable                            |
|                                 | Non-exposed | 466                           | 32            | 434                                      | Cumulative dose (mg/m\textsuperscript{3}) × year (41.4–94.43) | Not applicable                             | Not applicable                              |                                 |
| Zhang et al.\textsuperscript{31} | Exposed >30 mg/m\textsuperscript{3} | 411                           | 29            | 382                                      | Not applicable                           | Not applicable                             | Not applicable                             | Not applicable                            |
|                                 | Exposed <30 mg/m\textsuperscript{3} | 211                           | 5             | 206                                      | Not applicable                           | Not applicable                             | Not applicable                             | Not applicable                            |

DMF: N,N-dimethylformamide.

\textsuperscript{a}Different set up of the 2 × 2 contingency table due to matched pairs analysis.

\textsuperscript{b}Number of workers (N).

\textsuperscript{c}Calculated from the range \(((\text{max value}–\text{min value}/2) + \text{min value})\) of DMF exposure.

\textsuperscript{d}The remaining subjects showed normal findings.
sufficient quality. Liver function was carefully recorded in all included studies, while most studies have taken into account factors that could confound the relationship between DMF exposure and liver toxicity. In addition, a symmetrical (inverted) funnel plot indicated no publication bias (i.e., inclusion of selective publications). Thus, there is no reason to assume overestimation of the effect of DMF exposure on liver toxicity due to publication bias.

Studies included in meta-analysis

In a study conducted by Cirla et al., a significant increase in γ-GT in 25/100 exposed (DMF exposure levels ranged from 8 mg/m³ to 58 mg/m³) and only 10/100 controls (p < 0.01) was reported. Prevalence for higher serum levels of AST (9 vs. 3) and ALT (12 vs. 8) was not statistically different in the exposed group compared to the control group. Alkaline phosphatase (AP) values were normal in both groups. When subjects who had not modified their alcohol consumption upon working with DMF were considered, the effect was still evident.

High-exposure concentrations of DMF (75–180 mg/m³) were significantly related to increased levels of ALT (ALT ≥ 35 U/l), a result that remained the same even after the hepatitis B status was considered. This confirms the finding that a synergistic effect of DMF exposure and hepatitis B carrier status is not solely responsible for the high prevalence of abnormal ALT.

Another factory survey was conducted with 206 exposed workers and 142 non-exposed workers, in whom time-weighted average (TWA) exposure to DMF, haematology, serum biochemistry and subjective symptoms were investigated. In the exposed group, DMF exposure ranged from 3 mg/m³ to 27 mg/m³. Non-exposed workers served in the sections applying water/steam, nitrogen or hydrogen. In summary, the prevalence of borderline and abnormal cases when liver function indicators such as albumin, ASAT-ALAT, γ-GTP, ALP-LAP and LDH were examined among the exposed group was not different from that in the non-exposed group.

In a carefully conducted investigation of liver function in 75 workers in a synthetic leather factory, geometric mean levels of DMF in the air were approximately 20 mg/m³ with a span of exposure between 2 mg/m³ and 40 mg/m³. The control group consisted of 75 unexposed workers similar in age, sex, social status and residence. All mean serum ALT, AST, γ-GT and AP were significantly higher in the exposed group compared to the control group. Similarly, exposed workers were more likely (approximately 23%) compared to the control group (4%) to have abnormal transaminase values.

The effects of DMF exposure were investigated in 126 exposed workers (DMF air concentration ranged from less than 0.3 mg/m³ to 113.7 mg/m³) compared with 54 non-exposed workers, in combination with ethyl alcohol consumption. The results suggested a statistically significant toxic influence of DMF on liver function with alcohol having a synergistic effect. However, when there was no alcohol consumption for both groups, no significant differences of DMF exposure on liver enzymes were observed. These results should be interpreted with caution as the confounding effect of alcohol can only be analysed to a limited extent.

TWA of DMF was calculated in a study conducted by He et al., categorizing the exposed group into three subgroups with mean levels of exposure ranging from below 2.55 mg/m³ (low exposure/control) to about 36 mg/m³ (high group). The highest reported exposure was 199.80 mg/m³. The results showed that liver enzyme values were significantly increased with the amount of exposure; nevertheless, urinary AMCC concentration had greater potential to determine DMF-induced liver damage combined with elevated liver enzyme activities.

In a study by He et al., significantly abnormal mean serum activities of liver enzymes (ALT, AST and γ-GT) were reported for workers exposed with sub-threshold limit value (TLV) level (20 mg/m³) and γ-GT (8 h TWA) of the DMF exposure. The TWA concentration of the DMF was 18.6 mg/m³ (range 9.80–36.2 mg/m³), and the authors cautioned that the wide fluctuation indicated the possibility of
occasional overexposure. Moreover, 29% (21/72) versus 4% (3/72) of the exposed compared to the non-exposed workers had an abnormal liver function.

In a study conducted with workers from two factories, Kilo et al.\textsuperscript{40} divided the exposed workers into a group of low DMF exposure (<15 mg/m\textsuperscript{3}) or high DMF exposure (≥15 mg/m\textsuperscript{3}) based on the current OEL of 15 mg/m\textsuperscript{3} as proposed by the Scientific Committee on Occupational Exposure Limits.\textsuperscript{19} The range of exposure was between 0.08 mg/m\textsuperscript{3} and 46.9 mg/m\textsuperscript{3}. Between or within-group comparisons showed no significant increase for any of the liver function tests (LFT; ALT, AST, \(\gamma\)-GT). However, it was assumed that DMF could still cause certain alcohol intolerance reactions but seems to be in a dimension not influencing alcohol consumption behaviour.

In similar lines, Wu et al.\textsuperscript{41} found that higher liver injury rates were identified in DMF-exposed (levels ranged from 6.3 mg/m\textsuperscript{3} to higher than 30 mg/m\textsuperscript{3}) workers (\(n = 698\)) versus non DMF-exposed workers (\(n = 188\); 9.17% vs. 4.26%; \(p = 0.029\)) and in male versus female workers (11.4% vs. 3.2%; \(p < 0.001\)). The mean exposure was not reported. A significant dose–response trend in liver injury rates was identified among the four environmental exposure groups (\(p < 0.001\)) categorized based on worksites.

A study by Catenacci et al.\textsuperscript{42} investigated liver function (serum glutamate–oxalate transaminase (SGOT/AST), serum glutamate–pyruvate transaminase (SGPT/ALT), \(\gamma\)-GT and AP) in workers employed for at least 5 years in an acrylic fibre plant; no mention was made of exposure to other solvents. The first group of 28 subjects worked in the spinning department, where DMF exposure (8-h TWA) ranged from 12 mg/m\textsuperscript{3} to 25 mg/m\textsuperscript{3}, with a mean of 3 mg/m\textsuperscript{3}. The control group consisted of 54 subjects matched for age, smoking/alcohol consumption and history of liver disease, who had never been occupationally exposed to solvents. Mean serum values for SGOT/AST, SGPT/ALT, \(\gamma\)-GT and AP did not differ among the three groups and were within the normal ranges.

### Meta-analysis results

The quantitative section of this review summarizes the findings of 10 observational studies. In particular, the association between DMF exposure and liver toxicity in exposed compared to non-exposed workers was investigated firstly, in a dose–response subgroup analysis and, secondly, in an analysis in which all studies were analysed together regardless of the level of dosage.

The analysis by dosage stratification showed that there was no significant effect if the midpoint DMF exposure was ≤ 20 mg/m\textsuperscript{3} (Online Supplemental Figure S1; summary OR: 1.58, 95% CIs: 0.68–3.65, heterogeneity \(I^2\): 0%, \(p = 0.65\)). The summary OR was 1.24 (95% CIs: 0.43–3.53, heterogeneity \(I^2\): 0%, \(p = 0.60\)) when a cutoff value of ≤ 15 mg/m\textsuperscript{3} was chosen (data not shown).

A positive association between DMF exposure and liver toxicity was however observed (Online Supplemental Figure S2) when the midpoint DMF exposure was ≥ 21 mg/m\textsuperscript{3} and ≤ 25 mg/m\textsuperscript{3} (summary OR: 3.26, 95% CIs: 1.38–7.73, heterogeneity \(I^2\): 72%, \(p = 0.01\)). Similarly, a positive association was observed (Online Supplemental Figure S3) when the midpoint exposure was ≥ 26 mg/m\textsuperscript{3} (summary OR: 2.17, 95% CIs: 1.23–3.81, heterogeneity \(I^2\): 0%, \(p = 0.48\)).

In the overall analyses, when all studies with different dosages were combined (Online Supplemental Figure S4), a positive association between exposure to airborne DMF and liver abnormality was observed (summary OR: 2.30, 95% CIs: 1.53–3.47, \(I^2\): 34%, \(p = 0.13\)).

Sensitivity analyses on the aforementioned results did not change the pooled estimates. In detail, a leave-out-one-study sensitivity analysis showed no evidence that any of the studies included in the meta-analysis could be suspected of excessive influence on the overall estimate (See Online Supplementary Material 1). In addition, when we performed a meta-analysis leaving out the studies conducted in Asian populations, the pooled effect estimates attenuated but remained statistically significant with no considerable shift or change in heterogeneities or \(p\) values of the results (See Online Supplementary Material 2).

### Studies not used in the meta-analysis

Levels of serum hepatic enzymes in human populations occupationally exposed to DMF have been determined in some observational studies that were not eligible to be included into the meta-analyses but are nevertheless worth reporting. Two studies by Lauwerys et al.\textsuperscript{27} were carried out among workers exposed to DMF in an acrylic fibre factory. In the first study, several LFTs were performed on Monday and Friday morning on all control (\(n = 28\)) and exposed (\(n = 22\)) workers. The percentage of values exceeding slightly the upper normal limit as defined for an adult population and the mean value of the various parameters were not significantly different between the two groups. Likewise, there were no differences between the Monday and the Friday individual results between the exposed and the control groups. When the exposed workers were classified into two subgroups according to their integrated exposure to DMF vapour during the 5-day observation period (above or below 300 mg/m\textsuperscript{3} × h), no significant difference between the two subgroups was found. It was concluded by the authors that exposure to DMF vapour for 5 years at a level usually below 30 mg/m\textsuperscript{3} does not seem to entail a risk of liver cytolysis.

Exposure of DMF was measured in nine male workers,\textsuperscript{29} handling surface-treating agents containing DMF (levels ranged from 0 mg/m\textsuperscript{3} to 15.39 mg/m\textsuperscript{3}) for five consecutive days. The results suggested that LFTs (SGOT, SGPT, ALP,
γ-GTP of the workers conducted half-yearly for 3 years were in the normal range.

Redlich et al.\textsuperscript{43} suggested enzyme abnormalities, that is, elevated values (twice higher than the normal values observed for adults) of either AST or ALT in 35 of 46 (76\%) exposed workers. However, levels of DMF exposure were not mentioned in this study and therefore these results cannot be used to discern safe conclusions about liver toxicity.

Moreover, Yang et al.\textsuperscript{28} (only abstract available) suggested an increase in liver toxicity in 3/13 (23\%) of workers after a 15-min short-term exposure level. However, quantitative data on the DMF exposure levels were not described for each production zone and therefore the interpretation of these results is limited.

Likewise, personal exposure levels of DMF (levels ranged from 7.5 mg/m\textsuperscript{3} to 31.2 mg/m\textsuperscript{3}) in 10 workers for 3 years were measured in a study by Sakai et al.\textsuperscript{30} The results suggested that the mean levels of LFTs were within the normal levels.

Major et al.\textsuperscript{32} reported an increase in serum enzymes (significance not reported) in 26 workers exposed to 0.6–24 mg/m\textsuperscript{3} DMF with very high concomitant exposure to acrylonitrile (ACN). Six of the 26 (23\%) exposed subjects were hospitalized because of liver dysfunction which had developed due to inhalative exposure to DMF. However, these findings are of limited value for drawing conclusions over the effect of DMF exposure on liver function due to poor reporting and the high concomitance with ACN.

Abnormal LFTs were observed by Luo et al.\textsuperscript{44} in 24 of 65 (36.9\%) workers with high DMF exposure (>30 mg/m\textsuperscript{3}), 10 of 37 (27\%) workers with middle DMF exposure (15–30 mg/m\textsuperscript{3}) and 11 of 74 (14.9\%) with low DMF exposure (<15 mg/m\textsuperscript{3}). When compared with the workers having low DMF exposure, the adjusted ORs for abnormal LFTs were 1.62 (95\% CI: 0.61–4.28) for those with middle DMF exposure and 2.93 (95\% CI: 1.27–6.8) for those with high DMF exposure. A statistically significant linear trend between DMF exposure, the adjusted ORs for abnormal LFTs were 1.62 (95\% CI: 0.61–4.28) for those with middle DMF exposure and 2.93 (95\% CI: 1.27–6.8) for those with high DMF exposure. A statistically significant linear trend between DMF exposure and liver function due to poor reporting and the high concomitance with ACN.

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In a cohort study with a 4-year follow-up conducted by Qi et al.,\textsuperscript{26} workers exposed to a cumulative dose of DMF (annual DMF average in the workplace was 7.0 mg/m\textsuperscript{3}; 3.3–21.6 mg/m\textsuperscript{3}) were significantly more likely than the non-exposed workers to develop liver injury. The threshold of the cumulative DMF dose for liver injury was 7.30 mg/m\textsuperscript{3} in the year.

In another large cohort study conducted by the Leather Synthetic Enterprises in Jiangsu, China,\textsuperscript{31} 411 factory workers were exposed above the TLV (30 mg/m\textsuperscript{3}) and 211 workers were exposed below the TLV. The incidence of liver injury in the above the DMF TLV group was higher than in the below the DMF TLV group (6.3\% vs. 2.4\%). Moreover, the relative risk was almost three times higher in the above the TLV group (2.76; 95\% CI: 1.05–7.25) compared to the group exposed below the TLV.

**Discussion**

The present review and meta-analysis is the first to report on the relationship between DMF exposure and liver toxicity in populations of factory workers. Estimates of the risk of liver toxicity related to varying dosage of occupational DMF exposure were presented. We found no relationship between DMF exposure and liver function observed when the midpoint DMF exposure of workers was less or equal to 20 mg/m\textsuperscript{3}. However, a statistically significant effect of DMF exposure on liver function was observed when the midpoint DMF exposure was higher than 21 mg/m\textsuperscript{3}. Our finding suggestive of DMF-induced liver toxicity should be interpreted with caution. Elevated levels of liver enzymes can be caused due to several reasons, as discussed below, and findings showing statistically significant differences in liver enzyme levels when compared to control groups give no information on the potential biological pathway inducing liver toxicity.

Despite evidence from studies in occupational settings, suggesting that both acute and chronic DMF exposure, even below TLV levels, can lead to damage in the liver, underlying mechanisms by which DMF induces liver injury remain largely unknown. DMF can be easily absorbed by the dermal and respiratory systems. Its metabolism could lead to the depletion of the synthesis of glutathione in response to oxidative stress.

Some lines of evidence suggest that the effects of DMF on the liver may be enhanced in some special populations or by other hepatotoxins. For instance, significant associations have been observed between abnormal LFTs and hepatitis B virus (HBV) carriers, and between LFTs and increased body mass index (BMI), which suggested that HBV carrier status and increased BMI can have synergistic effects with DMF causing liver abnormalities.\textsuperscript{44}

Moreover, genetic polymorphisms of some related genes responsible for DMF metabolism could be responsible for discrepancies across different populations. CYP2E1 is the major enzyme for DMF metabolism. Some functional single nucleotide polymorphisms (SNPs) of CYP2E1 show large differences in minor allele frequency (MAF) in different populations. For example, the MAFs of SNPs (rs3813867 and rs2031920) associated with increased hepatocarcinogenic risk and the SNP (rs6413431) associated with alcohol-related hepatic disease\textsuperscript{45} show differences between Asians and Caucasians.\textsuperscript{46}

If this potential modifying effect was substantial, this would have been reflected in higher ORs for Asian compared to European studies. However, after excluding the Asian populations, we showed that the ORs remained high regardless. This suggests homogeneity between results, and the biological mechanisms and the size of the effect are likely to be similar for Asian and European populations,
at least across the studies of this systematic review. As ethnic background has not been shown to be a source of heterogeneity in this study, the Asian studies do indeed contribute to the evidence base. As such, information from Chinese studies should also be relevant for European workers.

Several other factors such as the role of gut microbiota, the disturbance of calcium homeostasis and apoptosis in hepatotoxicity of DMF have barely been investigated in humans.

In addition, a variety of often unknown confounding factors may influence the levels of liver enzymes. As the ‘normal’ values for liver enzymes are derived by definition for 95% of the ‘normal’ healthy population, liver enzymes exceeding the “normal” level may be observed even in healthy subjects. It has been earlier reported that 2–6% of asymptomatic individuals have higher levels of ALT, AST and \( \gamma \)-GT probably associated with alcohol consumption, asymptomatic hepatitis or drugs.\(^{47}\)

However, whether these factors play a causal role or just occur accompanied with the onset of liver injury induced by DMF remains to be elucidated in further human studies.

**Strengths and limitations**

In this meta-analysis, we used midpoint DMF exposure values as all of the studies reported minimum and maximum DMF exposure levels. Only, two studies reported the medians, five studies reported the means of the DMF exposure and no study reported sufficient data to recalculate study specific dose–response curves. A meta-analysis on medians or means may have led to selection bias due to the small number of studies that could provide such data. Nevertheless, we repeated the analysis using mean DMF values anyway. When the mean (available for five studies) DMF exposure values were \( \leq 15 \text{ mg/m}^3 \) or \( \leq 20 \text{ mg/m}^3 \), we again observed a non-significant association between DMF exposure and liver toxicity (summary OR: 1.33, 95% CIs: 0.82–2.17, heterogeneity = 0%, \( p = 0.87 \) and summary OR: 1.97; 95% CIs: 0.81–4.74, heterogeneity = 64%, \( p = 0.04 \), respectively). When we analysed mean values of DMF exposure higher than 15 mg/m\(^3\) (summary OR = 3.08; 95% CIs: 1.98–4.79, heterogeneity = 12%, \( p = 0.34 \)), we observed a significant association between DMF exposure and liver toxicity. We also repeated the meta-analyses using the medians (available for two studies) of DMF exposure. The relationship between median DMF exposure and liver toxicity was not significant (summary OR = 1.38; 95% CIs: 0.83–2.28, heterogeneity = 0%, \( p = 0.94 \)) when the median exposure level was \( \leq 15 \text{ mg/m}^3 \).

Nevertheless, the use of midpoint values should be interpreted with care. Overestimation compared to median values and both overestimation and underestimation compared to mean values can occur depending on the distribution of the exposure level of the individual study.

Populations with a high exposure at the upper end of the exposure distributions will lead to an overestimation of liver toxicity. We have shown, however, robustness of the analysis as the results were comparable when means, medians or midpoints were considered.

The high quality of almost all of the studies, the accountability of most common confounding factors and the low heterogeneity observed add to the strengths of this study.

In conclusion, based on the strengths of the methodology and the stability of the results confirmed via the sensitivity analyses, this meta-analysis shows no relationship between midpoint DMF exposure at a level below or equal to 20 mg/m\(^3\) and liver toxicity.

**Declaration of conflicting interests**
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