Original article

# Detection of DNA *adduct 8-hydroxy-2'-deoxyguanosine* (8-OHdG) as a toxicity bioindicator to the effects of nickel on Ni-Cr alloy prosthesis users

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A R T I C L E  I N F O

A B S T R A C T

Previous studies have suggested that exposure to Ni from Ni-Cr alloys can affect the human body through oxidative stress. The present study discusses the effect of nickel from Ni-Cr alloy prostheses on the formation of DNA Adduct 8-Hydroxy-2′-Deoxyguanosine (8-OHdG), evaluated based on creatinine and 8-OHdG concentrations in urine, determined with LC-MS/MS, for a Ni-Cr alloy user group and a never-user control group. The mean creatinine and 8-OHdG concentrations were not significantly different between the test groups, although highest levels were observed for the in the Ni-Cr user group. It is suggested that samples with relatively high creatinine and/or 8-OHdG levels are further studied in more detail for stability of concentrations and for the effect of contributing factors.

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## 1. Introduction

Forensic toxicological analyses are useful to determine the presence and concentration of potentially harmful substances, and to evaluate their nature or role in the human environment (Rai and Kaur, 2013; Kumar and Ganesh, 2016; SOFT/AAFS. Forensic Toxicology Laboratory Guidelines., 2006; Center and Simplified Guide, 2012; Gorea, 2009).

Nickel (Ni) can appear at low concentrations in air, soils, drinking water, and living organisms such as plants, particularly around nickel mines and some industrial environments (Ma et al., 2015; Milheiro, 2015). As metal, Ni is found in imitation jewelry, coins, and dental materials, and as compound residue in many everyday items such as food, detergents, soaps, cosmetics, and cigarettes (Milheiro, 2015; Calogiuri et al., 2016). In dentistry, Ni is used in alloys for space maintainers, brackets, fillings, and crowns (Kulkarni et al., 2016). In much of the world, Ni-containing cast alloys are common materials of fixed prosthodontics (Elshahawy and Watanabe, 2014; Chen et al., 2013).

The amount of Ni in dental alloys can be more than 60% (Kulkarni et al., 2016). Dental alloys containing nickel can corrode, thereby releasing metal ions. The ionic components released by Ni-Cr alloys are absorbed by the human body, either through the oral mucosa, digestive system, skin or respiratory tract, to become a part of the chemical environment of the cellular metabolism (Kulkarni et al., 2016; Uzun et al., 2014). Ni exposure can occur for short or prolonged periods of time, both at low and high concentrations (Hariyani et al., 2015). Orthodontic equipment has been reported to release 22–40 µg/day Ni (and 36 µg/day Cr) in vitro. This release is affected by salivary conditions, regional pH values, diet, smoking habits, chewing gum, oral hygiene, structure and composition of equipment, and mechanical and thermal loads (Milheiro, 2015).

Ni has a detrimental effect at the level of cells, tissues, organs and organisms, and nickel is also carcinogenic in humans (Amini et al., 2012; Keinan et al., 2010). Toxicity and carcinogenicity of some nickel compounds is related to uptake, transport, distribution, and retention at the cellular level (Keinan et al., 2010; Sun et al., 2013). Ni complexes in the form of arsenides and sulfides can be carcinogenic and mutagenic. Even at nontoxic concentrations, Ni can cause DNA changes mainly through alkaline damage and DNA cutting (Anand et al., 2015).

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The release of metal ions is characterized by a successive reduction of electrons from oxygen molecules to water in aqueous solutions. This will produce a series of reactive oxygen species (ROS) such as superoxide radicals ($O_2/HO_2$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals (OH) (Haohao et al., 2014). Ni is an active redox transition metal that can produce ROS and cause oxidative DNA damage, potentially contributing to the pathology of various diseases (Klaunig et al., 2011; Ni et al., 2014).

Unstable molecules (intermediates) of lipids and protein oxidation processes can react with DNA and form DNA adducts. These are compounds that can bind to DNA, cause mutations and trigger cancer (Rajalakshmi et al., 2015). Hydroxyl free radicals ($OH^-$) are considered as the main ROS that interact with DNA bases, deoxyribose and free nucleotides. There are at least 24 modified bases associated with identified ROS attacks (Klaunig et al., 2011). Of the modified bases, 8-hydroxy-2′-deoxyguanosine (8-OHdG) is the main adduct and is widely accepted as a marker of oxidative DNA damage and oxidative stress (Ni et al., 2014).

Although various types of DNA products appear during oxidative damage on DNA, particular interest is focused on nucleobase modulation and especially on abundant 8-OHdG lesions. They form in vivo and can be measured in cells after DNA hydrolysis to base components (Valavanidis et al., 2009). Direct 8-OHdG identification is often carried out with high performance liquid chromatography (HPLC, such as LC-MS/MS) (Dabrowska and Wiczkowski, 2017; Shrivastava and Gupta, 2012; Guo et al., 2016). The interest in the present case is mainly related to the effects exerted by dental Ni-Cr alloys, with grades designed to limit the dissolution of metal ions in the environment of oral fluids (Radev, 2012; Senkutvan et al., 2014; Mchouh, 2015; Castro et al., 2015; Milheiro et al., 2011). Studies on such release have indicated normal urinary Ni levels of about 4.5 µg/L in people with occupational exposure to nickel and about 9 µg/L in orthodontic patients. It has been suggested that dental Ni is more easily absorbed than Ni in foods that is combined in protein complexes by microorganisms (Chen et al., 2013; Milheiro et al., 2011; Al-Subari et al., 2013; Martinez-Zamudio and Ha, 2011). Ni can produce ROS through a range of Fenton, Fenton-like and Haber-Weiss reactions (Yadav and Sharma, 2016; Ezraty and Barras, 2016; Park et al., 2005; Peter et al., 2014). The impact of Ni and ROS would then depend on actual level of availability of the harmful constituents (Latvala et al., 2016; Goncalves, 2015; Manke et al., 2013). The level of urine 8-OHdG can be a good indicator of oxidative damage to the entire body's DNA. Urine is a convenient diagnostic biofluid in clinical practice, because it is sterile and easily obtained in large volumes without invasive methods (Guo et al., 2016; Akiibinu et al., 2016; Handayani et al., 2017; Inaba et al., 2011; Maddela et al., 2017; Devanshu et al., 2010).

Considering the potential negative effects of nickel in the body, we aimed to compare the users of Ni-Cr alloy prostheses and never-users in terms of the formation of DNA adduct 8-OHdG as a potential toxicity bioindicator.

## 2. Materials and methods

The present study was a descriptive cross-sectional analysis aiming to determine the formation of 8-OHdG in the subjects. The study group included 13 individuals of civilian flight personnel in Indonesia who came to the Aviation Health Center to conduct a complete routine health check, and clinically used Ni-Cr alloy prosthesis for a minimum period of 1 year. The control group consisted of 13 otherwise comparable individuals who did not use metallic prosthesis such as crowns, bridges, brackets, inlays, onlays, and removable dentures. The subjects had no smoking habit nor a history of cancer.

The variables to be measured are nickel in the Ni-Cr alloy prosthesis as an independent variable and the formation of DNA adduct 8-hydroxy-2′-deoxyguanosine (8-OHdG) as the dependent variable. The operational definition of nickel in prosthesis for Ni-Cr alloy materials is urine samples from prosthesis users stored in biomedical freezers at −20 °C. The operational definition of DNA adduct 8-OHdG formation is a modification of the guanine (G) base from deoxyguanosine caused by (OH•) to 8-OHdG, measured by looking at the peak formed equal to the chromatogram retention time of the standard 8-OHdG solution. The study received ethical approval of the Faculty of Dentistry, University of Indonesia Research Ethics Commission. It also received the permits to conduct research at the Aviation Health Center of the Ministry of Transportation of the Republic of Indonesia. The selected subjects meeting the inclusion criteria provided informed consent approval and filled in the personal data including name, address, age, type of dental and oral care used, length of use of the equipment, health status, smoking habits, and history of the disease. Then, urine samples were collected in a 15 cc falcon tubes, temporarily stored in a cooler box at 4 °C and then stored at −20 °C until the time of analysis.

Standard 8-OHdG solutions were prepared by dissolving 1 mg of 8-OHdG compound in 1000 µL of aquabidest, and diluting to concentrations of 50 ppb, 100 ppb, 250 ppb, 500 ppb, 1000 ppb, and 1500 ppb. Sodium acetate solution of 130 mml/L at pH 4.5 was prepared by dissolving 1.07 g sodium acetate salt in 90 mL aquabidest, then adjusting pH to 4.5 with the addition of 1 M HCl. The solution was then diluted with aquabidest to a volume of 100 mL. To prepare 25 ppm creatinine solution, 1 mg creatinine was dissolved in 1 mL 0.1 M HCl. The 25 ppm stock solution was made by dissolving 250 µL of 1000 ppm standard solution with 10 mL 0.1 M HCl. Standard solutions were made at concentrations of 1200, 1000, 800, 600, 400, 200, 100, and 50 ppb.

After all reagents were prepared, the samples were analyzed for 8-OHdG formation in urine using LC-MS/MS. Calibration for linearity of indicated 8-OHdG was carried out by comparison to each of the standard 8-OHdG solutions, in as much as 7 repetitions, with a linear fit with correlation $R^2 > 0.996$. Creatinine in urine was analysed using the *de Jaffe* method, to describe the normal function of kidneys by comparison to the normal limit range (according to WHO) of 0.3–3 g/L. Calibration for linearity of indicated creatinine was conducted as above for 8-OHdG, from creatinine standard concentrations of 50, 100, 200, 400, 600, 800, 1000, and 1200 mg/L. The solution measurement was carried out by UV-Vis spectrophotometer at a wavelength of 486 nm. For statistical analysis of the results, t-tests and Mann-Whitney tests were applied using SPSS version 20.

## 3. Results

The standard measurement results of 1500 ppb 8-OHdG was based on mass spectrometry (MS) precursor $m/z$ 284, appearing with retention time of 0.59 min, which is within the expected time. The optimum value obtained from the parameters of each mass spectrometer instrument is a good result for the fragmentation of 8-OHdG compounds in Q1 and Q3. Data from Q1 produced 284 precursor ions and was further fragmented into data from Q3, which produced the highest intensity product ion at 168 (Fig. 1).

LC-MS/MS validation of 8-OHdG compound gave a linear fit of $y = 6.0475x + 411.31$ with a correlation $R^2 = 0.9986$ (Fig. 2), which was better than the required minimum of 0.996.

Creatinine validation measurement using a UV-Vis spectrophotometer with wavelength of 505 nm gave a linear fit of $y = 0.0016x + 0.0572$ with correlation $R^2 = 0.9972$ (Fig. 3) that was better than the required 0.996.
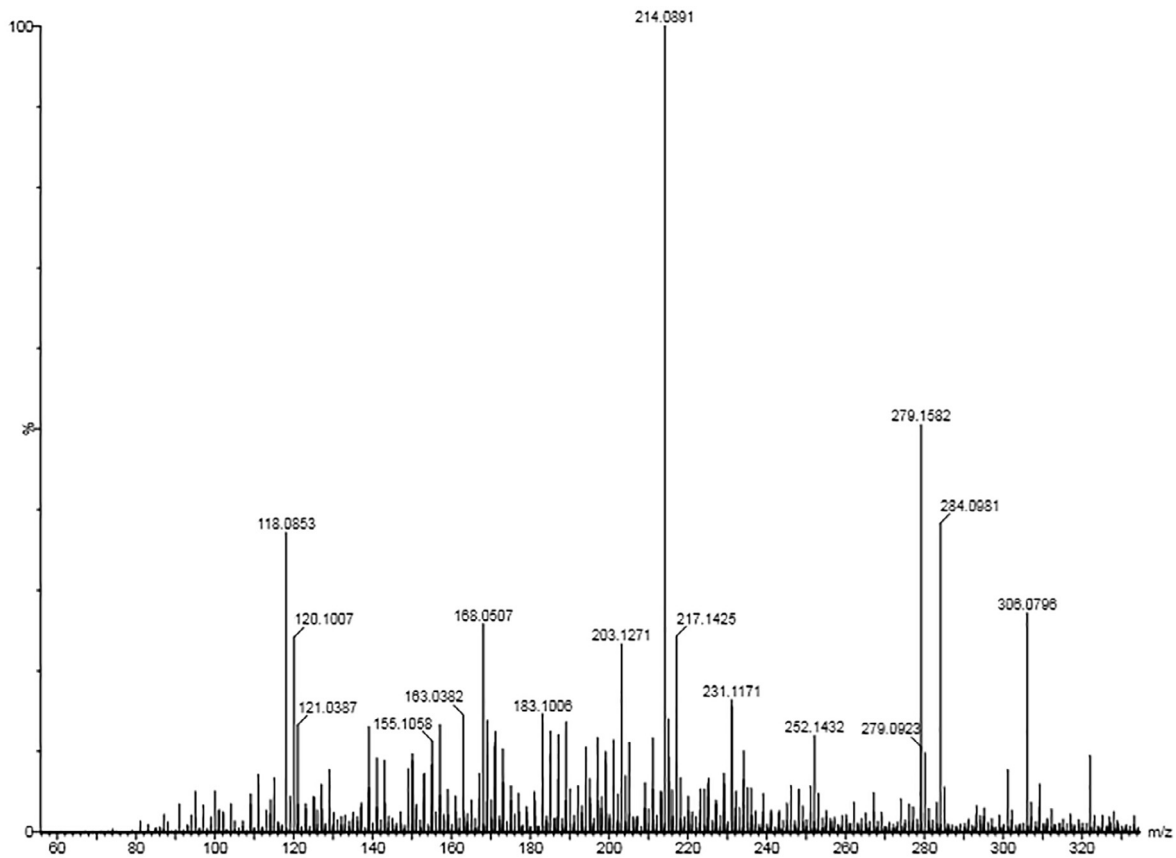
**Fig. 1.** Optimization of mass spectrometer chromatogram with 284 values from Q1 (precursor ions) and 168 values from Q3 (product ions).
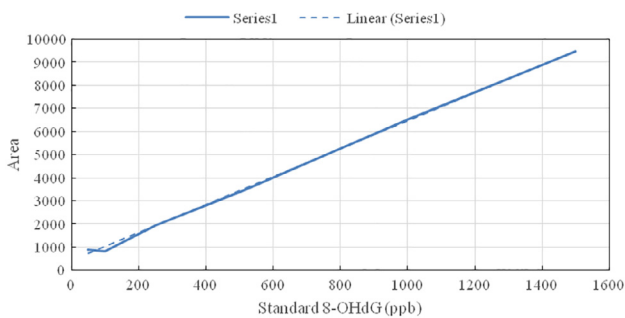

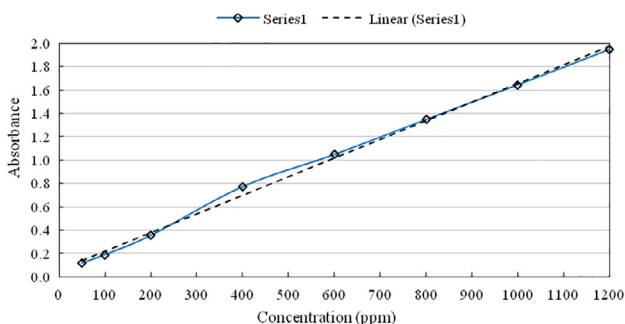
**Fig. 2.** 8-OhdG standard calibration curve.



**Fig. 3.** Creatinine standard calibration curve.

Table 1 and Fig. 4 show measured creatinine levels for the samples from the control group and the Ni-Cr alloy user group. The range of observed creatinine levels was 0.284 to 2.218 g/L in the control group, and 0.174 to 3.606 g/L in the Ni-Cr alloy group.
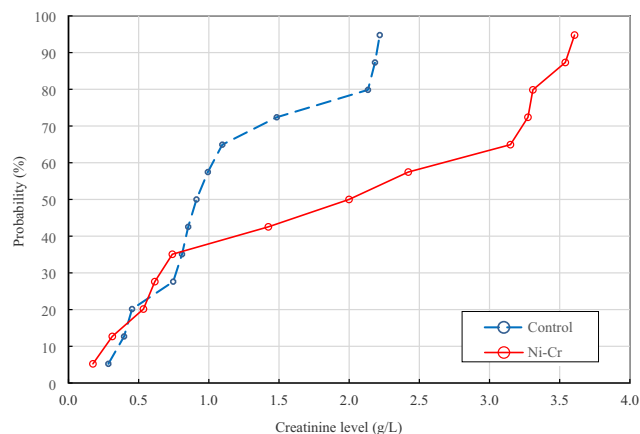
**Table 1**
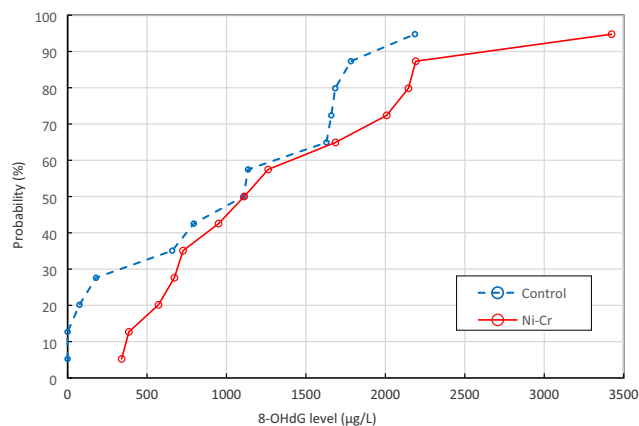Measured creatinine levels in control and Ni-Cr alloy user groups.

| Sample code | Creatinine level (g/L) | Sample code | Creatinine level (g/L) |
|---|---|---|---|
| Control 1 | 0.853 | NiCr 1 | 0.174 |
| Control 2 | 0.809 | NiCr 2 | 0.534 |
| Control 3 | 0.453 | NiCr 3 | 1.424 |
| Control 4 | 0.284 | NiCr 4 | 3.540 |
| Control 5 | 2.134 | NiCr 5 | 2.421 |
| Control 6 | 0.746 | NiCr 6 | 3.274 |
| Control 7 | 1.096 | NiCr 7 | 0.312 |
| Control 8 | 0.912 | NiCr 8 | 0.740 |
| Control 9 | 0.396 | NiCr 9 | 1.999 |
| Control 10 | 2.218 | NiCr 10 | 3.149 |
| Control 11 | 1.484 | NiCr 11 | 3.309 |
| Control 12 | 2.184 | NiCr 12 | 3.606 |
| Control 13 | 0.993 | NiCr 13 | 0.615 |

For one control group sample the observed creatinine concentration of 0.284 g/L was slightly lower than the lower limit of the normal range, while the maximum observed levels were well within the normal range (WHO standard: 0.3–3 g/L). In the Ni-Cr alloy user group one sample, at 0.174 g/L, was more clearly below the lower limit of the WHO range, but five samples showed creatinine concentrations above the upper limit of the WHO range. Comparison of creatinine concentrations by Mann-Whitney non-parametric test showed no significant difference (p = 0.249 > 0.05) between the control group and the Ni-Cr alloy user group.

Table 2 and Fig. 5 show the measurement results of 8-OHdG concentrations of the control and Ni-Cr alloy user groups. The observed range of 8-OHdG concentration was 0 to 2185.8 μg/L in the control group, and 340.2 to 3424 μg/L in the Ni-Cr alloy user group. The results of the independent t-test (Table 3) showed no statistically significant difference in the 8-OHdG concentrations between the control group and the Ni-Cr user group.

**Fig. 4.** Rank probability of occurrence for the measured creatinine levels in control and NiCr alloy user groups.



**Fig. 5.** Rank probability of occurrence for the measured 8-OHdG levels in control and NiCr alloy user groups.

## 4. Discussion

The measured creatinine and 8-OHdG levels exhibited relatively wide scatter both in the control group and in the Ni-Cr alloy user group, without statistically significant overall differences between the groups.

The observed creatinine concentration in the control group was generally within the expected range of the WHO standard (0.3–3 g/L), although one control sample showed a marginally lower concentration (0.284 g/L) than the minimum of 0.3 g/L (Table 1 and Fig. 4). Although (at 0.174 g/L) one sample in the Ni-Cr alloy user group also had a creatinine concentration lower than the standard minimum, this group included five samples (38.5% of the group) exceeding the expected maximum of 3 g/L. As the differences are much clearer between the samples showing highest peak creatinine concentrations, it therefore might be more appropriate to study the subjects with highest levels in more detail for the stability of the creatinine levels and further possible reasons for the observed high values (e.g. diet and alloy characteristics).

**Table 3**
Results of independent $t$-test between the groups on 8-OHdG concentration.

| Group | Mean (SD) | Median (minimum–maximum) | $p$-value |
|---|---|---|---|
| Control (n = 13) | 991.7 (765.8) | 1107.1 (0–2185.8) | 0.294 |
| NiCr (n = 13) | 1344.2 (904.4) | 1111.5 (340.2–3424.0) | |

The observed 8-OHdG concentrations in the control and Ni-Cr alloy user groups was generally rather similarly distributed, with high scatter and no statistically significant difference between the groups overall (Table 3 and Fig. 5). However, again some shift may occur at the highest observed levels, so that only one sample (7.7%) in the control group but four samples (30.8%) exceeded the 8-OHdG level of 2000 µg/L. As for creatinine, it could be of interest to conduct a follow-up study on the cases with highest observed levels of 8-OHdG regarding the stability of these levels in time. Consideration should be given on possible contributing factors such as the (measured) Ni content in the body, and other relevant

**Table 2**
Measured 8-OhdG concentrations.

| Sample Code | Dental Treatment | Smoking Habit | Duration of Use | 8-OHdG Level (µg/L) |
|---|---|---|---|---|
| Control 1 | None | No | Never | 658.3 |
| Control 2 | None | No | Never | 179 |
| Control 3 | None | No | Never | 0 |
| Control 4 | None | No | Never | 794.2 |
| Control 5 | None | No | Never | 2185.8 |
| Control 6 | None | No | Never | 1684.2 |
| Control 7 | None | No | Never | 0 |
| Control 8 | None | No | Never | 1783.1 |
| Control 9 | None | No | Never | 1629.1 |
| Control 10 | None | No | Never | 74.8 |
| Control 11 | None | No | Never | 1659.8 |
| Control 12 | None | No | Never | 1107.1 |
| Control 13 | None | No | Never | 1136.0 |
| NiCr 1 | Metal Crown | Sometimes | >10 years | 571.9 |
| NiCr 2 | Metal Crown | No | 18 years | 1111.5 |
| NiCr 3 | Metal Crown | Often | >20 years | 672.1 |
| NiCr 4 | Metal Crown | No | 2–3 years | 2145.1 |
| NiCr 5 | Metal Crown | No | 25 years | 726.5 |
| NiCr 6 | Metal Crown | No | > 10 years | 1262.3 |
| NiCr 7 | Metal Crown | Sometimes | 5 years | 385.5 |
| NiCr 8 | Metal Crown | No | 20 years | 340.2 |
| NiCr 9 | Metal Crown | No | 8 years | 2008.4 |
| NiCr 10 | Metal Crown | No | 4 years | 1686.0 |
| NiCr 11 | Metal Crown | No | 10 years | 3424.0 |
| NiCr 12 | Metal Crown | No | 20 years | 2191.5 |
| NiCr 13 | Metal Crown | Often | 4 years | 950.1 |

factors of the oral and cellular environment. Without considering the impact of individual factors it could be challenging to untangle the level of universality of the markers such as 8-OHdG.

Note also that when comparing seven highest creatinine levels in Table 1 and seven highest 8-OHdG levels in Table 2, six of same samples (Ni-Cr samples no. 4, 6, 9, 10, 11 and 12) are included in the list. This may further emphasize the importance of the samples with highest measured concentrations of creatinine and 8-OHdG. The available information on patient data is likely to set limitations to sampling and studies on the associated factors, because many endogenous and exogenous factors could affect the 8-OHdG levels (Klaunig et al., 2011; Pizzino et al., 2014).

## 5. Conclusion

In conclusion, the results suggest that while no significant differences were observed in the overall creatinine and in the DNA adduct 8-hydroxy-2′-deoxyguanosine (8-OHdG) concentrations between the control and Ni-Cr alloy user groups, the highest concentrations for both creatinine and 8-OHdG occurred in the Ni-Cr alloy users. Therefore, Ni from prosthetic devices may contribute to the metabolically active Ni load, when the conditions favor Ni dissolution and accumulation. It is suggested that cases with relatively high creatinine and/or 8-OHdG levels are longitudinally studied for stability of concentrations and contributing factors in more detail.

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