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Qualitative detection of formaldehyde in challani fish obtained from selected fish markets of Nagaon, Assam, India

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Abstract
Preservatives are used to increase the shelf life of any product for a very long time, yet they are extremely detrimental to human health when used in food. The use of preservatives like formaldehyde by fishermen and fish merchants for prolonged preservation of fish during sales and transportation has recently become problematic and threat to human health. Therefore, the goal of the current investigation was to determine the presence of formaldehyde in the fish sold at fish markets in Nagaon, Assam. The present investigation indicates the contamination of fish with formaldehyde in the local fish markets of Nagaon district. The fish that show the presence of formaldehyde are imported (challani) and out of the whole fish sample collection (challani/imported), *Labeo rohita, Catla catla* indicates the highest positive result, with 35.96% and 48.44% respectively from the 114 *Labeo rohita* to 64 *Catla catla* samples. Additionally, the results for *Ompok pabda, Pangasius pangasius, Hilsa ilisha*, and *Piaractus brachypomus* are all positive, with *Hilsa ilisha* showing a positive result of 100% from their whole sample collection. However, the local fish like *Labeo gonius*, *Cirrhinus reba*, *Ctenopharyngodon idella*, *Cirrhinus mrigala*, and *Hypophthalmicthys nobilis* fish species have shown 100% negative results for formalin detection, indicating that their samples are formalin-free. Since formaldehyde is harmful to human health, better methods and techniques should be adopted for the preservation of fish for commerce and storage. Strict regulations should be put in place to limit the use of these hazardous chemical substances.

Keywords Formaldehyde · Preservative · Fish market · Environment

1 Introduction

For the majority of people around the world, fish is regularly one of the most popular and affordable dietary sources of animal protein. Even in rural areas, fish is a very affordable and readily available source of animal protein for human consumption [1]. It is an excellent source of critical nutrients, including high-quality protein, lipids, vitamins and minerals (micronutrients), which are crucial for the world's food and nutrition security [2, 3] In terms of protein, fish is a key source. 15–20% of body weight is made up of proteins [4]. Omega-3 fatty acids, which are abundant in fish, have several vital roles in the body, including acting as regulators of heart rhythm, structural components of every cell in the body, precursors of eicosanoids [5]. Similarly, Omega-3, Docosahexaenoic Acid (DHA), and Eicosapentaenoic Acid, some important dietary supplements found in fish, can induce some specific protein-targeting biogenesis pathway in the cytosol, which can prevent many human disorders like coronary heart disease, liver problems, diabetes,

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cancer therapy, COVID-19 therapy, etc. [6]. India produces almost 7.96% of the world's fish production, making it the second-largest fish producer after China, and the total production was estimated at 14.73 million metric tonne during the financial year 2021–22 [2, 7].

Fish has long been a staple diet and part of social life for people in Northeast India, particularly in Assam. Despite having water resources that may be developed for fisheries, the state's fish output has not yet reached self-sufficiency [8]. Fish is a nutritious diet that is a key source of −3 PUFAs, minerals, and animal proteins for people all over the world [9]. Fish imports in the state of Assam marginally dropped throughout the course of the entire time, but fish output increased from the years 2008–09 to 2015–2016 (2.07 lakh tonnes to 2.97 lakh tonnes). This indicates that the state's fisheries are producing fish in sufficient quantities and making fish readily available to consumers. According to the Department of Fisheries, Assam records from 2015 to 16, the state's current per capita nutritional demand is 11 kg, compared to the state's reported fish intake of 9 kg. Consequently, throughout the period, a shortage of 0.34 lakh tonnes of fish was noted [10]. Importing fish is required to satisfy these criteria. In Assam, the cost of challani fish is less than that of fish raised locally. For instance, challani carp species like Catla and Rohu are 100–150 rupees cheaper per kilogram than local carp species. Fish business administration increased as a result of the high demand for fish and fish products. This benefits the economy of the nation. However, there is a chance that its quality will decline while being transported [11].

Since the commencement of time, food additives have been utilized in food production. They serve a purpose in edible uses to improve specific aspects including appearance, texture, flavor, or shelf life as a kind of food preservation. Food preservatives are widely used in the food industry because they can increase the shelf life of foods and thus the revenue of food processors. Despite the positive effects, there are a lot of food preservatives on the market that have been used properly by food handlers. However, some people have abused these preservatives by using them outside of the prescribed allowable dose or in banned ways [12]. Formaldehyde is one of the preservatives that have been excessively used.

Fish is a perishable commodity, though, so there is a chance that its quality would decline while being transported [11]. Fish and fish products are readily deteriorating and are impacted by pre-harvest and post-mortem variables, which lower their value. This is due to the abundance of endogenous enzymes and psychrophilic bacteria in fish bodies as well as the delicate structure of fish. Formalin is employed as a preservative when the point of distribution is remote from the location of the catch [13].

In processed food products, such as fisheries goods, preservation is a standard procedure. For chemical preservation, different chemicals like nitric acid, sulphur dioxide, benzoic acid, sorbic acid, acetic acid, citric acid, formalin, etc. are most commonly used. These preservatives have their own unique characteristics of action [14]. Among these chemicals, formalin is one of the most commonly used preservatives in the fishery industry. Formalin, a 37% formaldehyde solution, is a cheap and efficient preservative that enters the tissue quickly [15]. The chemical name for formaldehyde is HCHO, and it is a colorless toxic organic molecule. Formaldehyde in excess amounts in food materials is dangerous to people's health. According to the International Agency for Research on Cancer, formaldehyde is a Group 1 Carcinogen [16]. It is one of the most well-known and frequently used preservatives for keeping dead animal bodies, especially fish, for a long period in museums, laboratories, etc. Additionally, it is frequently utilized as an antiseptic in veterinary pharmaceuticals and biological products and in fungicides, textiles, and embalming fluids [17]. Due to its ability to create muscle stiffness, formaldehyde reacts simultaneously with proteins and subsequently helps to keep the freshness of fish [18]. Fish and seafood naturally contain formaldehyde. Trimethylamine oxide (TMAO), which helps in fish postmortem, is primarily broken down into dimethylamine and formaldehyde. Most marine fish contain TMAO [19]. When fish flesh ages and degrades, formaldehyde may be produced. Other biochemical events, such as the oxidation of lipids brought on by microbial activity, can also take place in addition to the enzymatic reaction that naturally forms formaldehyde in fish and seafood. This will eventually cause physical harm to fish or the production of chemical byproducts like biogenic amines or other undesirable substances [20].

The US National Toxicology Programme declared formaldehyde to be "known to be a human carcinogen" on June 10, 2011. When it comes into contact with tissues, formaldehyde irritates them. The most typical symptoms include increased tears and irritation of the eyes, nose, and throat. These symptoms start to appear at air concentrations of between 0.4 and 3 parts per million (ppm). At 20 ppm, formaldehyde is immediately lethal to human life and health. According to a significant study, asthma sufferers may be more vulnerable to the negative effects of breathed formaldehyde than non-asthmatics. Large doses of formaldehyde can cause excruciating pain, vomiting, and even death. If the skin comes into touch with a powerful solution of formaldehyde, it may get inflamed [15]. Fish that has been preserved using formalin may cause cancer in any organ, including the stomach, lungs, and respiratory system, due to the unregulated proliferation of cells [21].

A fish's quality and market value are significantly influenced by its freshness attribute. The issue and its potential adverse effects are gaining more and more attention from customers in the present day. There have been allegations that the supply chain for the fish in Assam's market has been contaminated with formalin. As a result, the goal of the current research was to determine the formaldehyde content of various significant and highly edible fishes from the Nagaon fish market using HiMedia's HiRapid Formalin Test Kit (K137).

2 Materials and methods

2.1 Sample collection

Whole or cut fish were used for collecting the fish samples. Samples collected from several local fish markets in the Nagaon district including Dimoruguri Market 1, Dimoruguri Market 2, Kechali Market, ITI Market, Panigaon Chariali Market, Borbazar, Majorati Chariali Market, Nowgong Girls College Market, Dhing Gate Market, Bebejia Market, Panikhaiti Market, and Sulung Market. Having just their names and the knowledge that they are readily available at the market and frequently consumed by the local population, fish samples were randomly collected of various size ranges from 0.5 kg to 5 kg weight.

Forty-one fish of six different species from Dimoruguri Market 1, Forty-two fish of six species from Dimoruguri 2, Thirty-four fish of four species from Kechali Market, Ninety-five fish of four species from ITI Market, five fish of one species from Panigaon Chariali Market, Eighty-two fish of 11 species from Borbazar, Two fish of one species from Majorati Chariali Market, Eight fish of one species from Nowgong Girls College Market, Two fish of one species from Dhing Gate Market, Four fish of one species from Bebejia Market, one fish of one species from Panikhaiti Market, and fourteen fish of four species from Sulung Market were collected on different days. Several fish samples, including *Labeo rohita, Catla catla, Piaractus brachypomus, Labeo gonius, Cirrhinus reba, Ctenopharyngodon idella, Cirrhinus mrigala, Hypophthalmichthys nobilis, Ompok pabda, Hilsa ilisha, Pangasius pungasius, Thunnini* and *Sardinella gibbose* had been collected from the selected fish markets. A total of 330 fish samples in all were collected to check for the presence of formalin.

2.2 Detection of formalin

In the collected fish sample, formalin presence have detected by using the Himedia FISH test Kit (K137-1KT) called CIF-Test developed by ICAR-CIFT, Kerala is used to check for the presence of the chemical preservative formalin. These kits were used for qualitative formaldehyde detection within two minutes. A drop of reagent was then applied to the paper strip after it had been swabbed into the fish's surface three to four times in various locations of the fish body surface (Gill, Head, Body and Fish Fin). The developed color was compared to the reference color chart provided in the kit box after 1.5–2 min of waiting for maximum color development. The formalin presence was assessed by following the instruction given in the Kit along with the methodology followed by Devaraj et al. [18].

2.3 Instructions for using the test kit

A reagent bottle, test strips, and a comparison chart are all included in the kit. There are enough supplies in each kit to complete 25 tests.

Step 1: Prepared Reagent F-2 before the test (Reagent should be used within 20 days of preparation).
Step 2: The paper strip from bottle F-1 is taken and rubbed it on the fish surface/ cut surface to wet the paper strip.
Step 3: A drop of Reagent F-2 was added to the swabbed paper strip.
Step 4: Checked for development within 2 min.

The fish is safe to eat since the yellowish colour on its stripes indicates that it does not contain formaldehyde or formalin. If the resultant colour is green or dark bluish, it means that formaldehyde or formalin is present, and the fish is not safe to eat. 4 ppm is the lower limit of detection for formaldehyde in the strip [18].

3 Results and discussion

For the formalin detection by using the Himedia FISH test Kit, fish samples were collected from selected fish markets of Nagaon between December 20, 2022, and June 06, 2023.The results are presented in Table 1.

It was observed that the investigation using the kit that not all fish species have formalin content on them. Some of them are free of formalin while others have formalin contamination. The total number of fish samples was 330, of which 231 were found to be negative. Of these, 123 were challani (imported) fish and 108 were local fish. There were 99 fish in all that tested positive for formalin detection; all of them were challani fish except the *Ctenopharyngodon idella,* local fish species commonly known as Grass carp which tested positive from Sulung Market. Table No. 2 lists the various fish species collected from selected markets and their formalin detection test results.

According to the findings of the current study, formalin was not found in all fish species such as *Labeo gonius*, *Cirrhinus reba*, *Cirrhinus mrigala*, or *Hypophthalmicthys nobilis*. Other fish species, however, have both positive and negative formalin test results, including *Labeo rohita*, *Catla catla*, *Ctenopharyngodon idella,Ompok pabda*, *Pangasius pangasius*, and *Piaractus brachypomus*. The fact that *Hilsa ilisha*, *Thunnini*, and *Sardinella gibbose* display all positive findings indicate that all of the fish tested positive for formalin during the test. (Table 2). The majority of the fish markets in Nagaon, as well as the samples of rohu and catla fish, had formalin-treated fish if we exclude the other formalin-negative samples (Fig. 1). The amount of formalin present is irrelevant; however, according to Food Safety and Standards Authority of India (FSSAI) regulations, the presence of formalin implies that fish have been unethically handled with formalin [22].

FSSAI guidelines state that only ice should be used to preserve fresh fish and shellfish. It is unethical to use materials other than ice to prolong the keeping quality. Fish is a nutrient-dense dietary source that has a number of health advantages. Consumers appreciate it greatly, and it is in high demand around the nation. Fish is not getting to consumers in top condition due to its high perishability, a lack of facilities such as a cold chain, and the unavailability of high-quality ice. The dealers are turning to the use of illegal chemicals such as formalin to lengthen the storage time in order to meet these demands since seafood is so expensive.

The extensive use of formalin for long-term preservation in various fruits, vegetables, fish, meats, and milk poses a serious hazard to human health. Although formalin has historically been employed in the business for a variety of purposes, some dishonest traders use those chemicals to preserve foods for people that are hazardous and dangerous to human health. The United States Environmental Protection Agency and the International Agency for Research on Cancer have categorized formaldehyde as a potential human carcinogen and a class 2A carcinogen, respectively.

In comparison to formalin-treated fish, the bacterial load in fresh fish is higher during storage and transit. Animal nutrition refers to substances that are not proteins but can be converted into proteins by ruminant stomach microorganisms as non-protein nitrogen compounds (NPN compounds). With longer ice storage times, NPN content in fresh fish gradually increased. On the other hand, during the same duration of storage, the NPN concentration of fish that had been formalin treated gradually dropped [23]. In other words, formalin extends a fish's shelf life.

Since formaldehyde is retained on every surface of the body, numerous experimental studies have documented the deadly effects of exposure to it. Formaldehyde produces formic corrosive when too much of it enters the bloodstream, which can quickly necroses cells in the liver, kidneys, heart and brain [24]. In experimental animals, formaldehyde causes a variety of harmful consequences. It is a strong irritant of the upper respiratory tract that is almost fully deposited in the rodent's anterior nasal cavity [25]. The nasal mucosa is where formaldehyde is metabolized and where it also interacts covalently with DNA, RNA, and protein. Even in small doses, prolonged dietary formalin consumption may be lethal [26].

Qualitative detection of formaldehyde and ammonia in fish and other seafood obtained from Chennai's (India) fish markets, Devraj et al., 2021 reported the presence of formalin by using the rapid detection kits called CIFTest [18]. Both marine and freshwater species of fish have formaldehyde in their flesh, according to detection of formaldehyde content in selected fishes from three different retail markets in Mumbai [16]. This can be the result of a natural process in the case of marine fish. However, it's presumably because of adulteration along the marketing chain in freshwater fish. As reported by Hossain et al., 2008, formaldehyde is also detected in some fresh Rui fish in Bangladesh, and this is due to the lack of fish on ice [27]. Joshi et al., 2015 reported on their work in which formaldehyde was quantified at selected fish from the wet markets of the Kathmandu valley using the Nash test in conjunction with spectrophotometric analysis [28]. Formalin contamination was discovered in a few of Bangladesh's fish markets in Tangail and Tongi towns earlier [29].

Table 1 Formalin detection in fish obtained from selected fish markets in Nagaon

Sl. no	Scientific name	Local/ challani (Type)	Date of screening/ date of sample collection	Collection site	No. of specimen	Result	
						Positive	Negative
1	*Labeo rohita*	Challani	20.12.2022	Dhing Gate Market	2	2	0
2	*Catla catla*	Challani	23.12.2022	Bebejia Market	4	1	3
3	*Labeo rohita*	Challani	26.12.2022	Major Ati Market	2	2	0
4	*Labeo rohita*	Challani	27.12.2022	Nowgong Girls College Market	8	1	7
5	*Labeo rohita*	Challani	28.12.2022	Panigaon Chariali Market	5	1	4
6	*Catla catla*	Challani	29.12.2022	Panikhaiti Market	1	0	1
7	*Labeo rohita*	Challani	08.01.2023	Dimoruguri 1 Market	9	3	6
8	*Catla catla*	Challani	08.01.2023	Dimoruguri 1 Market	11	4	7
9	*Piaractus brachypomus*	Challani	08.01.2023	Dimoruguri 1 Market	7	3	4
10	*Labeo rohita*	Challani	10.01.2023	ITI Market	10	4	6
11	*Catla catla*	Challani	10.01.2023	ITI Market	7	4	3
12	*Labeo rohita*	Challani	18.01.2023	ITI Market	12	4	8
13	*Catla catla*	Challani	18.01.2023	ITI Market	13	8	5
14	*Labeo rohita*	Challani	18.01.2023	Kechaali Market	6	3	3
15	*Labeo rohita*	Challani	24.01.2023	Kechaali Market	10	3	7
16	*Labeo gonius*	Local	24.01.2023	Kecha ali Market	4	0	4
17	*Cirrhinus reba*	Local	28.01.2023	Kecha ali Market	3	0	3
18	*Ctenopharyngodon idella*	Local	28.01.2023	Kecha ali Market	4	0	4
19	*Cirrhinus mrigala*	Local	28.01.2023	Kecha ali Market	2	0	2
20	*Cirrhinus reba*	Local	29.01.2023	Kecha ali Market	1	0	1
21	*Ctenopharyngodon idella*	Local	29.01.2023	Kecha ali Market	4	0	4
22	*Labeo rohita*	Challani	02.02.2023	ITI Market	14	8	6
23	*Cirrhinus mrigala*	Local	02.02.2023	ITI Market	5	0	5
24	*Ctenopharyngodon idella*	Local	02.02.2023	ITI Market	3	0	3
25	*Catla catla*	Challani	02.02.2023	ITI Market	12	5	7
26	*Hypophthalmicthys nobilis*	Local	07.02.2023	Dimoruguri 2 Market	9	0	9
27	*Cirrhinus reba*	Local	07.02.2023	Dimoruguri 2 Market	2	0	2
28	*Labeo rohita*	Local	12.02.2023	Dimoruguri 2 Market	3	0	3
29	*Labeo gonius*	Local	12.02.2023	Dimoruguri 2 Market	11	0	11
30	*Ctenopharyngodon idella*	Local	12.02.2023	Dimoruguri 2 Market	2	0	2
31	*Labeo rohita*	Challani	14.02.2023	ITI Market	8	2	6
32	*Hypophthalmicthys nobilis*	Local	15.02.2023	Dimoruguri 1 Market	5	0	5
33	*Cirrhinus reba*	Local	15.02.2023	Dimoruguri 1 Market	8	0	8
34	*Ctenopharyngodon idella*	Local	15.02.2023	Dimoruguri 1 Market	1	0	1
35	*Ctenopharyngodon idella*	Local	24.02.2023	Dimoruguri 2 Market	1	0	1
36	*Hypophthalmicthys nobilis*	Local	24.02.2023	Dimoruguri 2 Market	3	0	3
37	*Labeo rohita*	Challani	24.02.2023	Dimoruguri 2 Market	8	2	6
38	*Catla catla*	Challani	24.02.2023	Dimoruguri 2 Market	3	1	2
39	*Labeo rohita*	Challani	07.03.2023	ITI Market	2	2	
40	*Catla catla*	Challani	07.03.2023	ITI Market	9	4	5
41	*Pabda (Ompok pabda)*	Challani	07.03.2023	Borbazar	7	4	3
42	*Hilsa ilisha*	Challani	07.03.2023	Borbazar	5	5	0
43	*Hypophthalmicthys nobilis*	Local	24.03.2023	Borbazar	13	0	13
44	*Ctenopharyngodon idella*	Local	24.03.2023	Borbazar	5	0	5
45	*Cirrhinus reba*	Local	24.03.2023	Borbazar	11	0	11
46	*Labeo gonius*	Local	24.03.2023	Borbazar	4	0	4
47	*Cirrhinus mrigala*	Local	24.03.2023	Borbazar	4	0	4
48	*Labeo rohita*	Challani	24.03.2023	Borbazar	15	4	11

Table 1 (continued)

Sl. no	Scientific name	Local/ challani (Type)	Date of screening/ date of sample collection	Collection site	No. of specimen	Result	
						Positive	Negative
49	*Catla catla*	Challani	24.03.2023	Borbazar	4	4	
50	*Piaractus brachypomus*	Challani	24.03.2023	Borbazar	8	3	5
51	*(Koch) Pangasius pangasius*	Challani	24.03.2023	Borbazar	16	5	11
52	*Thunnini*	Challani	05.06.2023	Sulung Market	3	3	0
53	*Sardinella gibbose*	Challani	05.06.2023	Sulung Market	4	4	0
54	*Ctenopharyngodon idella*	Local	05.06.2023	Sulung Market	4	4	0
55	*Hilsa ilisha*	Local	05.06.2023	Sulung Market	3	3	0

Table 2 Showing the number of different fish species collected from the chosen fish market, as well as the presence of formalin or not

Number of samples	Local name (trade name)	Scientific name	Result	
			positive	Negative
114	Rou (Rohu)	*Labeo rohita*	41 (35.96%)	73 (64.04%)
64	Bhokua (Catla)	*Catla catla*	31 (48.44%)	33 (51.56%)
19	Kuhi	*Labeo gonius*	0	19(100%)
25	Bhagun (Reba carp)	*Cirrhinus reba*	0	25 (100%)
24	Grass carp	*Ctenopharyngodon idella*	4 (16.667%)	20 (83.3335)
11	Mirika (Mrigala)	*Cirrhinus mrigala*	0	11 (100%)
30	Bighead (Bighead carp)	*Hypophthalmicthys nobilis*	0	30 (100%)
7	Pabho (Pabda)	*Ompok pabda*	4(57.14%)	3(42.85%)
16	Koch (Pangas catfish)	*Pangasius pangasius*	5 (31.25%)	11 (68.75%)
8	Ilish (Hilsa)	*Hilsa ilisha*	8 (100%)	0
15	Rupchanda (Chinese pomfret)	*Piaractus brachypomus*	6(40%)	9 (60%)
3	Tuna	*Thunnini*	3(100%)	0
4	Goldstripe sardinella	*Sardinella gibbose*	4(100%)	0

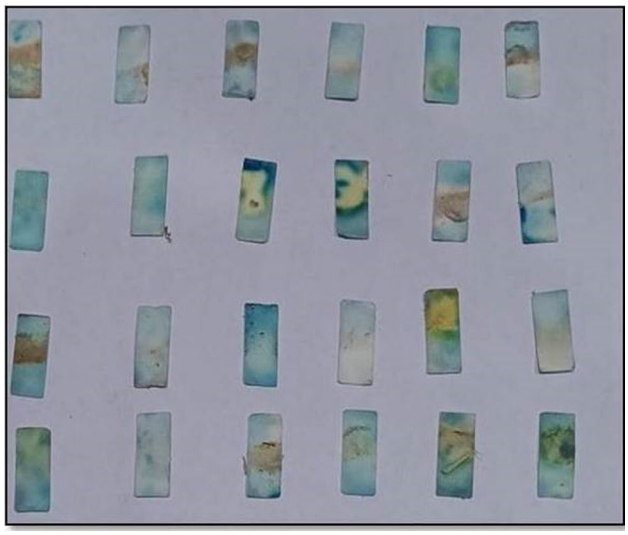

Fig. 1 Photograph of Representative results within two minutes after using the Himedia FISH test kit (K137-1KT)

4 Conclusion

Although preservatives have been used to extend the shelf life of foods since the beginning, it is unethical to use dangerous, carcinogenic substances like formalin (37% formaldehyde). At this point, it is quite dangerous that formalin detection was found positive in the local fish markets because fish is one of the main sources of protein and the most widely consumed affordable animal protein in society. People mostly belonging to the economically weaker section may prefer to purchase challani fish because of its low price and the shortage of local fish. Therefore, regular monitoring and proper testing should be done for the presence of formaldehyde/formalin along with other harmful chemicals for the greater interest of human health and society.

Author contributions Bhuban Chandra Chutia, Manash Pratim Borah, Lalit Mohan Goswami, and Sarat Borkataki have designed the entire study. Ujjal Bordoloi, Saswati Bharadawaj, and Meghna Borthakur have done the data collection. Priyanki Sharma and Jyotishmita Das screened samples. Ujjal Bordoloi prepared the first draft of the manuscript. Lalit Mohan Goswami and Manash Pratim Borah reviewed and edited the final version of the manuscript. All the authors reviewed and approved the final draft of the manuscript.

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Data availability All the data that support this study are included in the manuscript.

Declarations

Ethics approval and consent to participate All authors have read, understood and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the instructions for Authors.

Competing interests The authors declare that there is no financial or personal conflict of interests.

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