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Cannabinoid Concentrations Detected in Fatal Road Traffic Collision Victims Compared with a Population of Other Post Mortem Cases

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BACKGROUND: Acute cannabis consumption nearly doubles the risk of motor vehicle collision resulting in injury or death. Limited data have been published regarding the concentrations of cannabinoids associated with fatal road traffic collisions (RTCs), and these have not previously been compared to a population of other post mortem cases.

METHODS: We conducted analysis for cannabinoids [Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-THC, 11-nor-THC-9-carboxylic acid, cannabidiol, and cannabinol), drugs, and alcohol on consecutive fatal RTC cases (100) and non-RTC cases (114) from coroners' jurisdictions in London and southeast England and compared the data.

RESULTS: The incidence of cannabinoids detected in non-RTC and RTC cases was similar (25% vs 21%, $P = 0.44$), but THC was detected more frequently (90% vs 59%, $P = 0.01$) and at significantly higher concentrations in the cannabinoid-positive RTC cases than the non-RTC cases ($P = 0.01$). The distribution of non-RTC and RTC cases over 4 categories of THC concentration was significantly different ($P = 0.004$). There was no significant difference in the concentrations of other cannabinoids detected between the 2 groups. Cannabinoids were detected in more fatal RTC cases *(21)* than alcohol >80 mg/dL *(17)*. Detection of other drugs was low compared to cannabis and alcohol.

CONCLUSIONS: These first data on the concentrations of cannabinoids in the post mortem blood of fatal RTC victims compared with a population of other routine coroners' cases highlight the importance of specifically measuring THC concentrations in the blood to aid interpretation of post mortem cases where cannabis may be implicated.

Cannabis is the most prevalent drug used across the UK and worldwide *(1, 2)*. After alcohol (ethanol), it is the most frequent drug detected in victims in fatal road traffic collisions (RTCs)[3] in the United States, Australia, the UK, and many European countries *(3–5)*.

Evidence suggests that acute cannabis consumption nearly doubles the risk of motor vehicle collision resulting in injury or death *(6)*. Ingestion of cannabis impairs driving skills and reduces reaction times, road-tracking performance, performance in divided attention tasks, and hand-eye coordination. Users often slow their driving speed and take fewer risks, which may reflect overcompensation for perceived impairment *(7)*. Alcohol and drug ingestion by passengers may contribute to road traffic collisions, for example, by distracting a driver or promoting risk-taking behavior, particularly in younger victims *(8)*.

For occasional cannabis users, a blood concentration of 3.5–5 μg/L for the primary psychoactive component of cannabis, Δ^9-tetrahydrocannabinol (THC), has been suggested to result in impairment comparable to a blood alcohol concentration of 50 mg/dL (the blood alcohol limit for driving in most European countries) *(9)*. Drivers with THC in their blood are more likely to be responsible for a fatal crash, and the accident risk increases significantly when the THC concentrations are >5 μg/L *(10–12)*.

Interpretation of cannabinoid concentrations in post mortem blood can be difficult. Frequent users of cannabis may have detectable concentrations of THC in their blood for several hours or even days after their last use of cannabis *(13–15)*. A recent study showed THC to

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[3] Nonstandard abbreviations: RTC, road traffic collision; THC, Δ^9-tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-Δ^9-tetrahydrocannabinol; THC-COOH, tetrahydrocannabinol-9-carboxylic acid; CBD, cannabidiol; CBN, cannabinol.
Previous presentations: Some of these data were presented at the annual conference for the International Association of Forensic Toxicologists (TIAFT), November 2014, Buenos Aires, Argentina.

be present for up to 1 month of monitored abstinence *(16)*. Although it has been reported that neurocognitive impairment can persist for up to 28 days of monitored abstinence *(17)*, detection alone of cannabinoids in the blood does not demonstrate acute use or probable impairment. Second, post mortem redistribution of THC must be taken into consideration. Few data have been reported on post mortem blood THC concentrations in fatal RTC victims *(10–12;18;19)*. Of the studies previously reporting on the incidence of cannabis in fatal RTCs in the UK *(19–22)*, only 1 reported THC concentrations and did so for just 2 cases *(19)*. The remaining 3 studies did not distinguish between blood and urine results. Two of the studies reported outside of the UK were crash-responsibility studies in which the THC concentrations in drivers considered culpable were compared with those of drivers not considered culpable *(11, 12)*. These studies found that THC concentrations >5 μg/L significantly increased the risk of being involved in a fatal accident.

The incidence of cannabinoid detection in fatal RTC victims may simply reflect the high use of cannabis in the general population, rather than a causal link. Establishing a control population for post mortem studies is extremely difficult, since it is not possible to take post mortem samples solely for research purposes; samples may be collected only to assist in establishing the cause of death *(23)*. No previous study of cannabinoid detection has compared the incidence of cannabinoid detection in victims of fatal RTCs to a relevant non-RTC post mortem population.

The objectives of our study were (*a*) to compare the prevalence of cannabinoid detection and the concentrations of cannabinoids detected in post mortem blood from victims of fatal RTCs to the prevalence and concentrations detected in post mortem blood from other routine coroners' cases, and (*b*) to compare the prevalence of cannabinoid detection with that of other drugs and alcohol in post mortem blood from victims of fatal RTCs.

Materials and Methods

STUDY DESIGN

The Human Tissue Act 2004 gives coroners consent only to conduct analysis relevant to the investigation into the cause of death *(23)*. The analyses carried out were therefore determined on a case-by-case basis. The analyses specifically requested by the coroner or pathologist (via the sample request form) for each case were taken into account, and the age of the victim and the types of samples submitted were also considered. The analyses included gas chromatographic measurement of alcohol (ethanol) in blood, urine, and/or vitreous humor *(24)*; a general GC-MS screen of blood for drugs including unknown, licit, and illicit drugs *(25)*; a specific screen for morphine by Cozart® Forensic Microplate Enzyme Linked Immunoassay; and a 2-dimensional GC-MS screen and quantification for cannabinoids in blood *(26)*, which included analysis of THC, 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), cannabidiol (CBD), and cannabinol (CBN). The limit of detection for all analytes for the 0.5-mL sample analyzed was 0.5 μg/L, and the limit of quantification was 0.5 μg/L for THC, 11-OH-THC, and CBN and 1.0 μg/L for CBD and THC-COOH. A cannabinoid-positive case was defined as any case in which ≥1 of the analytes was present above the limit of detection. Urine was also screened for illicit drugs *(27)*. Quantification or confirmation of any drugs was carried out according to the UK and Ireland Association of Forensic Toxicologists' guidelines *(28)*.

STUDY POPULATION

The Toxicology Unit analyzes samples from coroners' jurisdictions across London and southeast England. This study took place between February 2011 and March 2013.

For the non-RTC group, we included consecutive coroners' cases that were not fatal RTC victims who had analysis of cannabinoids conducted as part of the requested routine analysis. A total of 114 of these consecutive non-RTC cases were analyzed for cannabinoids.

We considered all fatal RTC cases received during the study period. We analyzed 100 samples from victims of fatal RTCs for cannabinoids.

For both groups, all cases where the victim was >70 years old were excluded, because the incidence of illicit drug use in the >70-year age group is relatively low *(29)*, and it is unusual for illicit drug use analysis to be requested for cases in that age group.

ETHICS APPROVAL

Blood samples (ante mortem and post mortem) were collected by pathologists and submitted for toxicological analysis at the request of coroners as part of the investigation into the death. Approval for the use of the data generated from this analysis was granted by South West London Research Ethics Committee 1 (reference 11/LO/0033).

STATISTICAL ANALYSIS

We used the χ^2 test to assess for differences in the incidence of cannabinoids between the RTC and non-RTC groups and the incidence of THC between the cannabinoid-positive cases in the RTC and non-RTC groups. Previous studies vary in their estimations of cannabinoid detection in victims of RTCs. Sample size calculations suggested that 106 individuals were required in each group for a power of 80% and significance level (α) of 0.05, to determine a 3-fold greater incidence of cannabinoid detection in the experimental group.

We used the independent samples median test to analyze for differences in median age between the non-RTC and RTC group and between the cannabinoid-positive and cannabinoid-negative cases for each group.

We performed analyses to test for significant differences between the RTC and non-RTC groups for the distribution of post mortem blood concentrations of cannabinoids. The data were assessed and found not to follow a normal distribution, and therefore the nonparametric Mann–Whitney *U* test was used. We also assessed differences in the distribution of non-RTC and RTC cases between the categories of THC concentration with the Fisher exact test. A *P* value ≤0.05 was considered significant for all tests. IBM SPSS Statistics Version 20 was used for all analyses and verified by an independent statistician (Paul Bassett, Statsconsultancy, Amersham, UK).

CATEGORIZATION OF THC CONCENTRATIONS FOR INTERPRETATION

We grouped the cannabinoid-positive cases into 4 categories according to the measured THC concentration. The categories were chosen on the basis of evidence in the literature regarding concentrations associated with crash risk and driver culpability and impairment *(9–12)*: category 1, THC was not detected; category 2, THC concentration <3.5 μg/L; category 3, THC 3.5–5 μg/L; and category 4, THC >5 μg/L.

Results

DEMOGRAPHICS AND CLASSIFICATION OF NON-RTC CASES AND FATAL RTC CASES

The non-RTC and RTC cases had approximately equal numbers of males tested in each group (89 and 88, respectively). There were approximately twice as many females tested in the non-RTC group compared with the RTC group (25 and 12, respectively).

The age range tested for non-RTC cases was 16–68 years, with a median age of 40 years, and for the RTC cases was 12–69 years with a significantly lower median age of 35 years ($P = 0.03$).

The non-RTC cases were classified according to the case history received and were representative of the routine cases normally submitted by coroners for analysis. The RTC cases were classified as pedestrians, drivers, or passengers and according to vehicle type, if applicable. The classification of non-RTC cases and RTC cases is shown in Supplemental Fig. 1, which accompanies the online version of this article at http://www.clinchem.org/content/vol61/issue10.

Table 1. Summary of results of cannabinoid analyses.

Case description	Non-RTC	RTC
Analyzed for cannabinoids[b]	114 (100)	100 (100)
Positive for any cannabinoid	29 (25)	21 (21)
Males	23 (26)	19 (22)
Females	6 (28)	2 (17)
Age, years	39 (18-60)	24 (16-57)[c]
Positive for THC	17 (59)	19 (90)[d]
Positive for 11-OH-THC	20 (69)	15 (71)
Positive for THC-COOH	29 (100)	21 (100)
Positive for CBD	2 (7)	3 (14)
Positive for CBN	5 (17)	5 (24)
Positive for other illicit/licit drugs	17 (59)	1 (5)
Positive for alcohol >80 mg/dL	5 (17)	4 (19)
Case types		
Found dead	15	50
Drug/alcohol related		
Hanging	6	20
Other/unknown	3	10
Other[e]	2	7
Fall	2	7
Collapsed	1	3
Drug/alcohol related		
Other/unknown	0	0
Train death	0	0
Motorcyclist	9	43
Pedestrian	4	19
Car driver	4	19
Car passenger	3	14
Pedal cyclist	1	5
Driver other[f]	0	0
Pillion passenger	0	0

[a] Data are n, n (%), or median (range).
[b] Types of samples analyzed: ante mortem blood, 15; post mortem femoral vein blood, 196; post mortem heart blood, 2; and post mortem cavity blood, 1.
[c] Lower median age for RTC cases compared with non-RTC cases ($P = 0.03$, independent samples median test).
[d] Higher incidence of THC for RTC cases compared with non-RTC cases ($P = 0.01$, χ^2 test).
[e] Includes died in hospital, 2; multiple injuries, 1; light aircraft crash, 1; jumped from height, 2; carbon monoxide poisoning, 2; and set self on fire, 1.
[f] Includes driver lorry, driver van, driver quad bike, driver in control of car being towed, driver mobility scooter.

RESULTS OF CANNABINOID ANALYSIS

A total of 114 non-RTC samples and 100 RTC samples were analyzed for cannabinoids. A summary of the results is shown in Table 1. Cannabinoid-positive RTC cases had a significantly lower median age than cannabinoid-positive non-RTC cases (24 vs 39 years, $P = 0.02$). There was no difference in the median age between cannabinoid-positive and -negative non-RTC cases (39 vs 42 years, $P = 0.133$).

Table 2. Cannabinoid concentrations (μg/L) detected in post mortem blood from non-RTC and RTC cases.

Cannabinoid	Positive samples, n	Range[a]	Median
Non-RTC			
THC	16	<LOQ to 8.5	2.6
11-OH-THC	19	<LOQ to 5.7	1.8
THC-COOH	28	<LOQ to 94.8	7.3
CBD	2	<LOQ	NA[c]
CBN	5	<LOQ to 5.4	2.0
RTC			
THC	18	0.7 to 69.5[b]	4.2
11-OH-THC	14	<LOQ to 74.3	2.2
THC-COOH	20	1.8 to 220.5	11.9
CBD	3	<LOQ	NA
CBN	5	<LOQ to 1.1	0.5

[a] Limit of quantitation (LOQ) 0.5 μg/L for THC, 11-OH-THC, and CBN and 1.0 μg/L for THC-COOH and CBN.
[b] Greater distribution of THC for RTC cases compared with non-RTC cases ($P = 0.01$, Mann Whitney *U* test).
[c] NA, not available.

The cannabinoid-positive RTC cases had a lower, although not significant, median age than the cannabinoid-negative RTC cases (24 vs 36 years, $P = 0.05$).

There was a similar incidence of cannabinoids detected in non-RTC cases compared with RTC cases (25% vs 21%, $P = 0.44$), but the psychoactive component of cannabis, THC, was detected more frequently in the cannabinoid-positive cases from the RTC group than in those from the non-RTC group (90% vs 59%, $P = 0.01$). There was no significant difference in the incidence of 11-OH-THC between the non-RTC and RTC groups (69% vs 71%, $P = 0.85$). Additionally, all cannabinoid-positive cases in both groups were positive for THC-COOH. CBD was detected in only 2 non-RTC and 3 RTC cases. CBN was present in 5 cases in each group. A similar number of cannabinoid-positive non-RTC cases (n = 5) and RTC cases (n = 4) had a blood alcohol concentration >80 mg/dL, the current UK drunk driving limit.

COMPARISON OF CANNABINOID CONCENTRATIONS IN POST MORTEM FEMORAL VEIN BLOOD SAMPLES FOR NON-RTC AND RTC GROUPS

Ranges and median concentrations for all cannabinoids detected in the post mortem femoral vein blood samples from the non-RTC and RTC groups are shown in Table 2. Of the 24 cannabinoid-positive cases in the non-RTC group, 16 were positive for THC, with a median concentration of 2.6 μg/L (range <0.5–8.5 μg/L), and only 2 cases had concentrations >5 μg/L. Of the 20 cannabinoid-positive cases in the RTC group, 18 were positive for THC, with a median concentration of 4.2 μg/L (range 0.7–69.5 μg/L), and 8 cases had concentrations >5 μg/L.

The concentrations of THC detected in the cannabinoid-positive fatal RTC victims had a greater range and were significantly higher than those detected in non-RTC individuals positive for cannabinoids ($P = 0.01$). There was no significant difference in the distributions of 11-OH-THC or THC-COOH concentrations detected between the 2 groups ($P = 0.35$ and $P = 0.12$, respectively), but a similar trend was observed, with the highest concentrations detected within the RTC group. The distribution of THC, 11-OH-THC, and THC-COOH concentrations for both groups of cases is shown in Fig. 1. Because CBD and CBN were detected in only a small number of cases, the distributions were not compared.

The distribution of THC concentrations was also compared between RTC drivers (i.e., not including passengers or pedestrians) and the non-RTC group (Fig. 2). Although there was no longer a statistically significant difference in the distribution of THC concentrations between the 2 groups ($P = 0.07$), the trend of higher THC concentrations in the driver population was still evident.

CATEGORIZATION OF CANNABINOID-POSITIVE CASES FOR INTERPRETATION

There was a significant difference between the distribution of non-RTC and RTC cases within the 4 categories ($P = 0.03$). The non-RTC cases were mostly classified into categories 1 and 2, with only 4 cases in categories 3 and 4. The RTC cases were spread across all categories, with the highest numbers in categories 2 and 4. The categorization for the non-RTC and RTC cases is shown in Fig. 3, and the RTC cases are summarized in Table 3.

DETECTION OF ALCOHOL AND DRUGS IN THE RTC GROUP

No drugs were detected in 62 of the RTC blood samples analyzed, and ≥1 drug was detected in 38 of the RTC blood samples analyzed (62% and 38%, respectively). The most common drug detected was cannabis. Twenty-one blood samples were positive for cannabinoids, none of which were positive for any other drugs. The number of RTC cases positive for cannabinoids was greater than those with an alcohol concentration >80 mg/dL (17 cases). Drugs associated with emergency treatment were detected in 7 cases. Cocaine was the next most common finding, detected in only 5 cases. The prevalence of detection of all drugs is summarized in online Supplemental Fig. 2.

Discussion

We describe the first comparison of cannabinoid concentrations detected in post mortem blood from fatal RTC

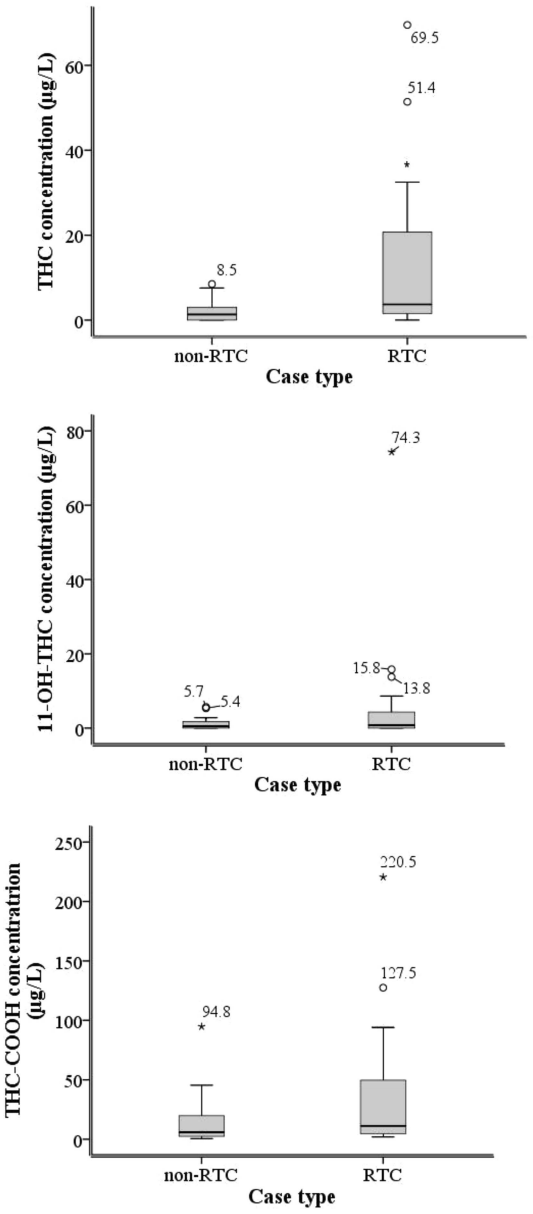

Fig. 1. Boxplot diagrams displaying the median and interquartile range of THC, 11-OH-THC, and THC-COOH concentrations detected in the cannabinoid-positive victims of fatal RTCs and non-RTC cases.

Only the distribution of THC concentrations was significantly different (*P = 0.01) for RTC cases compared with control cases. Individual numbers represent outlier values.

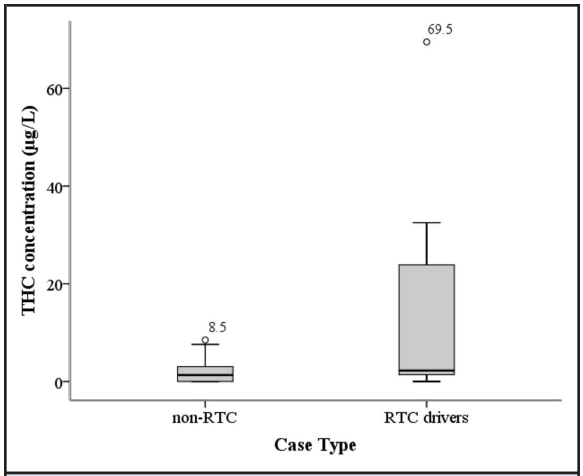

Fig. 2. Boxplot diagram displaying the median and interquartile range of THC concentrations detected in the cannabinoid-positive victims of fatal RTCs and non-RTC cases.

There was no significant difference in the distribution of THC concentrations (P = 0.07) for RTC driver cases compared with RTC cases. Individual numbers represent outlier values.

victims with those detected in a non-RTC post mortem group and the prevalence of cannabis compared to alcohol and other drugs in fatal RTC victims.

Our initial analysis of RTC victims included drivers, passengers, and pedestrians, because whether they were under the influence of alcohol or drugs may be of importance to the circumstances surrounding the RTC. This could be either directly, by being in charge of a vehicle, or indirectly, by being a passenger who distracts a driver or a pedestrian who walks in front of a moving vehicle. Unfortunately, further circumstantial details regarding victim behavior, driver culpability, or how long after the accident the blood sample was collected were not available for the RTC cases; such details would greatly aid the evaluation of these sorts of data.

Our data show that although the incidence of cannabinoids was similar between the 2 populations, THC concentrations were significantly higher in the RTC victims. The concentrations of 11-OH-THC and THC-COOH were not significantly different between the 2 groups but showed a similar trend of higher values in the RTC group. This highlights the importance of measuring THC concentrations in fatal RTC victims rather than just reporting the presence of cannabinoids.

CBD and CBN were detected in only a small number of cases in both groups. It has been suggested that CBD and CBN may be suitable markers for recent ingestion *(30)*. All but 1 of the CBD- and CBN-positive cases had THC concentrations >3.5 µg/L, the suggested lower limit of impairment proposed to correlate with a blood alcohol concentration of 50 mg/dL *(9)*.

Equating impairment to blood cannabinoid concentrations is not straightforward: a clear dose–response relationship has not been established, unlike for alcohol *(31)*. The pharmacology of cannabis makes it difficult to

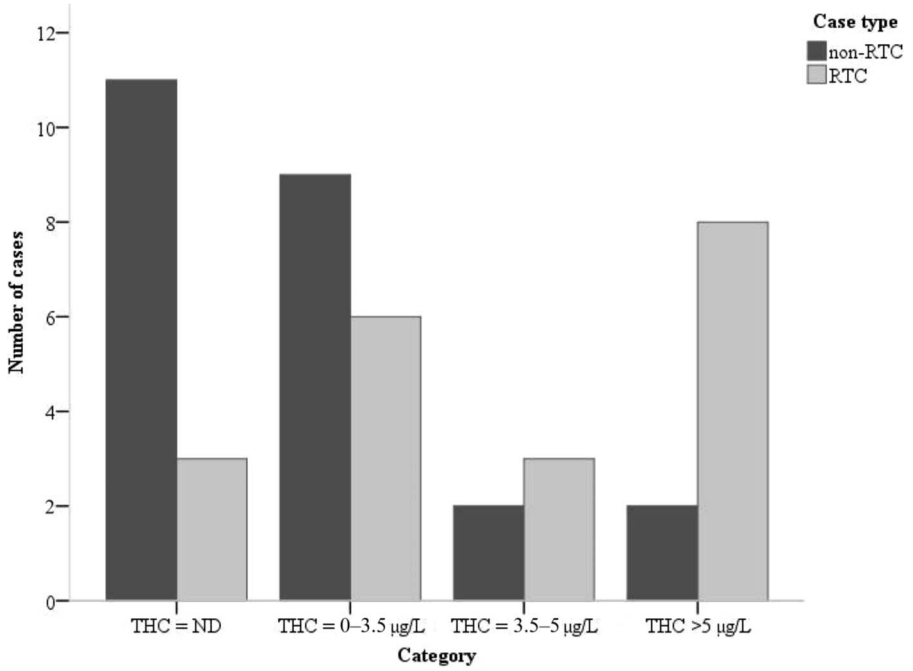

Fig. 3. Non-RTC and RTC cases grouped into distinct categories for interpretation.

interpret cannabinoid concentrations, both in life and in post mortem blood samples. First, blood concentrations measured in live individuals may not necessarily reflect recent ingestion if the person is a chronic user of cannabis. THC concentrations >1 μg/L in blood can be detected in chronic cannabis users up to 7 days after onset of monitored abstinence *(15)* and at lower concentrations for up to 1 month *(16)*. Second, post mortem blood concentrations may differ from those at the time of death due to post mortem redistribution. Because THC is stored within body tissues, after death, it will leach back out into the blood of chronic users, leading to increased concentrations of THC at the time of sampling versus at the time of death. However, there is recently published preliminary data suggesting that in 3 deaths in which both ante mortem and post mortem blood samples were available, concentrations of THC were lower in the post mortem peripheral blood samples than the ante mortem samples *(32)*. These limitations make it particularly important to establish the range of THC concentrations observed in other types of post mortem cases where the presence of cannabis is thought to be incidental, to distinguish higher concentrations in cases where cannabis may be implicated in the death, e.g., in fatal RTCs.

By categorizing the RTC cases according to previous interpretation of THC concentrations cited in the literature *(9–12)*, we attempted to distinguish cases in which impairment with cannabis was more likely to be a factor from other RTC cases where this was unlikely. However, such categorizations should be treated with caution because impairment cannot be conclusively demonstrated. The cases in category 1 were very unlikely to have been under the influence of cannabis at the time of death. Those in category 2 were unlikely to have been substantially impaired by cannabis at the time of death because the concentrations of THC detected were lower than 3.5 μg/L. The cases listed in group 3 may have suffered from low-level impairment, similar perhaps to a blood alcohol concentration of 50 mg/dL, and those in category 4 may have suffered from more severe impairment.

Concentrations up to 5 μg/L were observed in the non-RTC cases, where the finding of cannabis was incidental. Given the possibility of post mortem redistribution, these cases must be interpreted with caution. The final category contained 8 RTC cases in which the THC concentration detected was likely associated with more severe impairment compared with the other categories, on the basis of reports by Drummer et al. *(11)* and Laumon *(12)*. Of these cases, 5 victims were in charge of a motorcycle or car. Impairment from use of cannabis should be considered in these cases, although this must be assessed in the context of the available facts relating to the accident. The remaining 3 cases were passengers. Although the presence of cannabinoids in the blood of these victims may not be directly involved in the fatal accident, these passengers were under the age of 25 years, and

Table 3. Concentrations of cannabinoids detected in cannabinoid-positive post mortem blood specimens from RTC victims.

	Cannabinoid concentrations, μg/L					Blood alcohol concentration,	
Case	THC	11-OH-THC	THC-COOH	CBD	CBN	mg/dL	Type of RTC
R1	ND[a]	ND	2.5	ND	ND	145	Driver, SV, no seatbelt, driving at speed
R2	ND	ND[b]	9.8	ND[b]	ND[b]	<10	Motorcyclist at speed, struck other vehicle
R3	0.7	<LOQ	3.4	ND	ND	<10	Pedestrian hit by car
R4	1.0	ND	1.8	ND	ND	<10	Motorcyclist, collided with HGV
R5	1.4	ND	3.6	ND	ND	<10	Motorcyclist, SV, collided with CR
R6	1.7	0.8	10.4	ND	ND	<10	Cyclist, accidental fall onto pavement
R7	1.8	ND	5.8	ND	ND	<10	Pedestrian ran in front of bus, suicide note at home
R8	1.9	0.6	2.1	ND	ND	<10	Driver car, head on collision with a transit van
R9	2.6	0.7	16.5	ND	ND	<10	Motorcyclist, collision with car, cannabis at scene
R10	3.5	0.9	8.2	ND	ND	<10	Motorcyclist, collision with car
R11	3.9	ND	10.8	<LOQ	<LOQ	<10	Pedestrian hit by car while crossing road
R12	4.5	15.8	45.9	ND[b]	ND[b]	243	Pedestrian was in road, hit by car
R13	5.4	1.2	11.9	ND	ND	<10	Passenger rear, SV
R14	15.1	3.0	27.8	<LOQ	ND	<10	Passenger rear, SV
R15	17.6	74.3	220.5	<LOQ	<LOQ	<10	Motorcyclist, head on collision with car
R16	23.9	1.8	16.6	ND	ND	<10	Motorcyclist, collided with another vehicle
R17	26.5	2.2	53.5	ND	0.8	117	Driver, collided with oncoming van
R18	32.5	5.7	94.1	ND	1.1	<10	Motorcyclist overtaking, hit by oncoming vehicle
R19	51.4	8.7	73.4	ND	ND	<10	Passenger rear, SV, driver collided with CR
R20	69.5	13.8	127.5	ND[b]	ND[b]	116	Driver, SV, no seatbelt

[a] ND, not detected (limit of detection 0.5 μg/L); LOQ, limit of quantitation 0.5 μg/L for THC, 11-OH-THC and CBN and 1.0 μg/L for THC-COOH and CBD; NDD, no drugs detected; SV, single vehicle accident; CR, central reservation; HGV, heavy goods vehicle.
[b] Limit of detection 0.5 μg/L.

passengers in this age group under the influence of drugs or alcohol can often be a contributory factor in a fatal RTC *(8)*. Analyzing our data without including passengers and pedestrians shows a similar trend for increased THC concentrations in RTC drivers compared with the non-RTC group, although this effect did not achieve statistical significance.

Cannabinoids were detected in more fatal RTC cases *(21)* than alcohol >80 mg/dL *(17)*, and the incidence of other drugs was low compared to cannabis and alcohol. In 2 of the cases in which the concentration of THC was >5 μg/L, alcohol was also present at concentrations >80 mg/dL. Evidence shows that the combination of alcohol and cannabis significantly increases the risk of causing an accident *(33)*. The final decision on the involvement of cannabis in a fatal RTC would be made by the coroner in view of all other evidence relating to the death.

New drugged driving legislation came into force in the UK in March 2015, aimed at making roads safer and reducing accidents and deaths on the roads. The new legislation is similar to "per se legislation" that exists in other European countries and many US states *(34, 35)*. This is either implemented as a zero tolerance approach, whereby the lowest concentrations that can reliably be determined by a laboratory's analytical methods are used, or with cutoff concentrations determined by taking into account the effects of a particular drug. These cutoff concentrations range from 2 to 5 μg/L for THC in the blood *(36, 37)*. The limit set in the UK for cannabis is 2 μg/L THC in the blood. The data described here can be compared to similar data following a period of implementation of the law to assess if legislation is being effective at reducing deaths on the roads due to cannabis use.

Our data show a comparable prevalence of cannabis in the non-RTC and RTC groups but suggest that higher concentrations of THC are present in fatal RTC victims. These are the key data that need to be investigated further. Given the restrictions imposed by the Human Tissue Act, only post mortem samples submitted for routine analysis can be used. This limits the scope of the design of the study, because it was not possible to specifically select cases to include in the non-RTC group. It was impossible to eliminate bias because by the very nature of cases where

toxicology is requested, there may be a greater likelihood of detection of drug use. Also, these individuals will tend to have a younger age distribution than the general population and therefore may be more likely to use cannabis. However, there is no other appropriate comparable dataset available in which the results are likely to be similarly affected by post mortem redistribution of drugs, a key factor in interpreting post mortem cannabinoid concentrations.

Our data are, as far as we are aware, the first comparison of 2 distinct groups of post mortem cases where cannabinoid concentrations are compared between a group in which cannabis may be implicated in death and a group in which cannabis is thought to be incidental.

Further studies like this, alongside comparisons of ante mortem vs post mortem THC concentrations, will help further understanding of the impact that post mortem redistribution has on interpretation of THC concentrations in deaths where cannabis may be implicated. Our work supports the idea that cannabis use plays a role in fatal RTCs. However, given the mixed roles of our participants in these RTCs (driver, passenger, pedestrian), further work is required to determine whether driving under the influence of cannabis should be a priority focus point for legislation, and the utility of current and future laws regarding cannabis use and driving.

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