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ORIGINAL ARTICLE

RISK FACTORS, ERYTHROCYTE ACETYLCHOLINESTERASE INHIBITION, AND SELF-REPORTED SYMPTOMS OF PESTICIDE INTOXICATION AMONG FARMERS IN THAILAND: A CROSS-SECTIONAL STUDY

Ekarat Sombatsawat[1], *Wattasit Siriwong*[2], *Sitthichok Puangthongthub*[1]

[1]Industrial Toxicology and Risk Assessment Graduate Program, Department of Environmental Science, Faculty of Science, Chulalongkorn University, Bangkok, Thailand
[2]College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand

ABSTRACT

Background. Organophosphate and carbamate pesticides are widely used, and their adverse health effects remain a serious problem.

Objectives. This investigation aimed to describe risk factors, erythrocyte acetylcholinesterase (AChE) inhibition, and self-reported symptoms and to derive an association between influence factors and erythrocyte AChE inhibition among farmers in Thailand.

Methods. A cross-sectional study was conducted on 71 farmers from August to October 2022. General characteristics and pesticide exposure factors were elicited via a questionnaire-based interview. The erythrocyte AChE inhibition was assessed using the EQM Test-mate Cholinesterase (Model 400) instrument. Data were presented descriptively and analyzed statistically using *Chi*-square and binary logistic regression.

Results. Most farmers were over 50 years old and had an abnormal body mass index (BMI) without alcohol consumption and smoking. Aprons (18.31%) and protective eyewear (12.68%) as personal protective equipment (PPE) were found to be used less often. The level of hemoglobin-adjusted erythrocyte AChE (Q) was considered normal when it was 59.15% and abnormal when it was 40.85%. Self-reported symptoms were confirmed to be associated with lower erythrocyte AChE levels. The *Chi*-square analysis showed that shortness of breath, irritation, headache, dizziness, sleep fragmentation, and memory problems were significantly associated with erythrocyte AChE ($p < 0.05$). The bivariate analysis revealed that farmers who consumed alcohol while using pesticides (mixing, loading, and spraying) (OR=35.821, 95% CI=4.591–279.490), who did not wear a mask while using pesticides (OR=11.898, 95% CI=1.061–133.440), and who did not wear boots while using pesticides (OR=0.166, 95% CI=0.031–0.890) had an increased likelihood of having a severe inhibition of erythrocyte AChE.

Conclusions. These findings suggest that the promotion of risk prevention practices must be imposed on appropriate pesticide handling and PPE use among farmers.

Key words: *pesticides, erythrocyte acetylcholinesterase, self-reported symptoms, farmers*

INTRODUCTION

Pesticides are chemicals highly applied in agricultural sectors to control crop pests and boost agricultural production. In particular, organophosphate (OP) and carbamate (CA) pesticides are most widely used and are likely to become a major public health problem among farmers all over the world [1]. The primary pesticide-consuming countries include China, the USA, Argentina, India, Japan, Canada, Brazil, France, Italy, and Thailand [2]. The World Health Organization (WHO) has pointed out that pesticide poisoning is the most significant cause of severe toxicity and death from acute poisoning, with more than 300,000 deaths annually [3]. Farmers are normally exposed to pesticides during application via skin contact, inhalation, and ingestion. These chemical compounds inhibit erythrocyte acetylcholinesterase (AChE) enzymes and endocrine-disrupting substances [4]. Moreover, their toxicity interferes with the normal function of acetylcholine hydrolysis, a necessary task for synaptic response and an essential neurotransmitter in the autonomic and central nervous system [5], and these manifestations

Corresponding author: Sitthichok Puangthongthub, Industrial Toxicology and Risk Assessment Graduate Program, Department of Environmental Science, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, +66 2 218 5189, e-mail: sitthichok.p@chula.ac.th

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depend on the dose and the route of exposure involved in the occurrence [6]. The inhibition of cholinesterase activity can produce relatively mild and nonspecific symptoms of each physiological system, such as respiratory, gastrointestinal, urinary, exocrine gland, eye, skin, muscle, and nervous systems. The clinical diagnosis of pesticide poisoning is difficult; therefore, erythrocyte AChE activity can be used as a biological marker to confirm the toxins or residues or accumulation of certain pesticides in the body [7].

In Thailand, the number of pesticide poisoning patients has been increasing steadily. The Bureau of Occupational and Environmental Diseases (BOED) reported that the number of patients with pesticide poisoning in 2016 was 8,689 cases and in 2017 it was 10,312 cases, with an average annual incidence rate of 14.47 per 100,000 population and 17.12 per 100,000 population, respectively [8]. Important factors that lead to pesticide poisoning are farmers having low income, low education level, inaccessible information on pesticide safety, inadequate storage practices, poor maintenance of spraying equipment, and lack of personal protection during pesticide spraying [9]. Moreover, previous studies have shown that spraying frequency, spraying duration, working experience, and personal protective equipment (PPE) application are associated with neurobehavioral performance [10]. These might result in short-term or long-term effects on health illnesses, such as running nose, shortness of breath, anxiety, vomiting, hypersalivation, stomachache, diarrhea, blurred vision, headache, memory problem, dizziness, and unconscious [4]. The risk factor identification of pesticide poisoning is valuable for farmers to decline the number of poisoning cases. However, it is difficult to generalize and compare the findings with the differentiation of sociodemographic characteristics, supervisory mechanisms, use of mix-pesticides, exposure level, and geographical behaviors among the farmers. The objectives of this study were, therefore, to describe pesticide handling behaviors, erythrocyte AChE inhibition, and factors influencing pesticide exposure and to derive an association between influence factors and erythrocyte AChE inhibition among farmers in Thailand.

MATERIAL AND METHODS

Population and sampling

A cross-sectional study was conducted in the Phimai district, Nakhon Ratchasima province, Thailand, from August to October 2022, a season when pesticides are highly used. The study participants were 71 farmers who met the eligibility criteria and were recruited using the simple random sampling technique. The inclusion criteria were registered farmers in this study area who were over 18 years old (both male and female) and normally used OP and CA pesticides. The exclusion criteria were individuals with a history of diabetes disease, liver failure, myocardial infarction, malnutrition, and skin disorders related to sweating. Ethical approval for this experimental protocol was obtained from the Research Involving Human Research Subjects, Chulalongkorn University (COA no. 157/65). Participants signed an informed consent form before participating in the study.

Data collection

Data were collected through questionnaire-based interviews by researchers and a trained research assistant. Individual characteristics included gender, age, education, body mass index (BMI), alcohol consumption, smoking, farm size, working experience, working behaviors, and PPE application. The intoxication symptoms related to the respiratory, gastrointestinal, urinary, exocrine gland, eye, skin, muscle, and central nervous systems were self-reported with “No” or “Yes” answers. Blood samples of 10 μL per farmer were collected. Before blood collection, farmers were asked to wash their hands with soap and a medical technologist cleaned their fingers with alcohol. After that, the medical technologist used a needle to puncture their fingers. The first drop of blood was removed, and the second drop was collected using a capillary tube. The EQM Test-mate Cholinesterase Test System (Model 400) was used to test the erythrocyte AChE inhibition based on the manufacturers’ standard methodology [11]. The hemoglobin-adjusted erythrocyte AChE activity (Q) was measured in U/g Hb and was classified into two categories by the Tropical Pesticides Research Institute (2000): 1) inhibition if the activity is ≥24.5 U/g Hb and 2) severe inhibition if it is <24.5 U/g Hb [4].

Data analysis

Data were analyzed by the IBM Statistical Package for Social Sciences Licensed, Version 22 (IBM Corp.). All variables were presented in number (n), percentage (%), minimum (min), maximum (max), mean, median, and ±standard divination (±SD). Continuous variables were tested for distribution using the Kolmogorov–*Smirnov* test. A *Chi*-square test was used to find associations between variables and erythrocyte AChE activity. Binary logistic regression analysis was used to examine the association between influence variables and erythrocyte AChE activity presented with a crude odds ratio (OR). Variables with a *p*-value of <0.20 in the *Chi*-square test were included in the model. A *p*-value of <0.05 was considered statistically significant.

RESULTS

The individual characteristics of the farmers are presented in Table 1. The majority of participants were male (60.56%) and over 50 years old (64.79%). Most of them graduated from primary school (83.10%) with an abnormal BMI (67.61%). Farmers with habits of alcohol consumption and smoking accounted for 28.17% and 22.54%, respectively. About 61.97% of the farmers had farms of less than 12.25 acres, and 53.52% of them had work experience of over 11 years.

Of the subjects, 92.96% said that they washed their hands before eating and drinking after applying pesticides, 87.32% reported that they washed clothes by separating work clothes from normal clothes, and 84.51% revealed that they took showers with soap or cream immediately after using pesticides. Only a few of the interviewed farmers smoked while using pesticides (mixing, loading, and spraying) (8.45%) and dug a hole to bury a bottle of pesticides used (5.63%), as presented in Table 2. A total of 90.14% of farmers used masks, long sleeve shirts, and long pants during pesticide application, while only 18.31% and 12.68% of them used aprons and protective eyewear, respectively (Figure 1).

Table 3 describes the erythrocyte AChE inhibition (hemoglobin-adjusted) of OP and CA pesticides in farmers who applied them. About 59% of them had erythrocyte AChE with an inhibition level (≥24.5 U/g Hb) and 41% had a severe inhibition level (<24.5 U/g Hb).

As shown in Table 4, the highly reported symptoms by farmers were memory problems (64.79%) in the central nervous system, muscle fatigue (61.97%) in the muscle system, irritation (54.93%) in the eye system, and skin rash/itching (49.30%) in the skin system. The analysis (*Chi*-square) of association between self-reported symptoms and erythrocyte AChE showed

Table 1. Individual characteristics (n = 71)

Individual characteristics	n	%
Gender		
Female	28	39.44
Male	43	60.56
Age		
≤50 years	25	35.21
>50 years	46	64.79
Mean ± SD = 51.65 ± 6.31; median = 53.00; min–max = 32–59 years		
Education		
Primary school	59	83.10
Secondary and high school	11	15.49
Diploma	1	1.41
BMI		
Normal (18.50–22.99 kg/m^2)	23	32.39
Abnormal (underweight <18.50 and overweight ≥23.00 kg/m^2)	48	67.61
Alcohol drinking		
No	51	71.83
Yes	20	28.17
Smoking		
No	55	77.46
Yes	16	22.54
Farm size		
<12.25 acres	44	61.97
≥12.25 acres	27	38.03
Mean ± SD = 12.78 ± 29.07; median = 63.29; min–max = 1.19–50.96 acres		
Working experience		
<12 years	33	46.48
≥12 years	38	53.52
Mean ± SD = 13.32 ± 9.08; median = 12.00; min–max = 2–40 years		

Table 2. Working behaviors (n = 71)

Working behaviors	No	Yes
	n (%)	n (%)
Reading the label of pesticide products before use	17 (23.94)	54 (76.06)
Using the recommended amounts of pesticides	22 (30.99)	49 (69.01)
Washing hands before eating and drinking after applying pesticides	5 (7.04)	66 (92.96)
Taking a shower with soap or cream after using pesticides	11 (15.49)	60 (84.51)
Washing clothes by separating work clothes from normal clothes	9 (12.68)	62 (87.32)
Washing equipment after use	48 (67.61)	23 (32.39)
Storing pesticide products at home	14 (19.72)	57 (80.28)
Storing application equipment (sprayer) at home	12 (16.90)	59 (83.10)
Digging a hole to bury a bottle of pesticide used	67 (94.37)	4 (5.63)
Drinking while using pesticides (mixing, loading, and spraying)	56 (78.87)	15 (21.13)
Smoking while using pesticides (mixing, loading, and spraying)	65 (91.55)	6 (8.45)

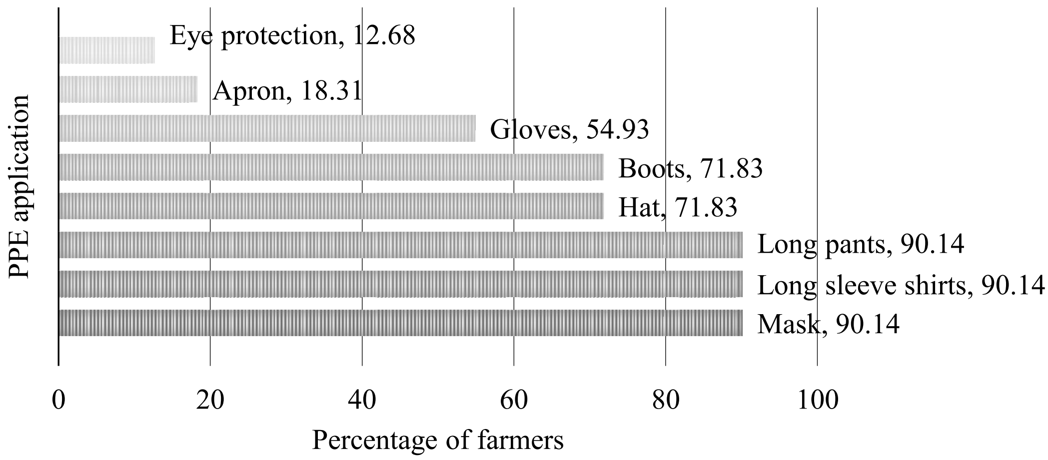

Figure 1. Personal protective equipment (PPE) application (n = 71)

Table 3. Categories of OP and CA pesticide intoxication (n = 71)

OP and CA pesticide intoxication	n	%
Hemoglobin-adjusted erythrocyte AChE inhibition		
Inhibition (≥24.5 U/g Hb)	42	59.15
Severe inhibition (<24.5 U/g Hb)	29	40.85
Mean (±SD) = 32.35 (±29.07); min–max = 3–129 U/g Hb		

that erythrocyte AChE was significantly associated with shortness of breath ($x^2 = 4.53$, $p = 0.03$), irritation ($x^2 = 2.94$, $p = 0.04$), headache ($x^2 = 6.46$, $p = 0.01$), dizziness ($x^2 = 8.24$, $p = 0.00$), sleep fragmentation ($x^2 = 3.43$, $p = 0.04$), and memory problem ($x^2 = 5.58$, $p = 0.01$).

According to the binary logistic regression analysis of each variable with erythrocyte AChE, farmers who consumed alcohol while using pesticides (mixing, loading, and spraying) (OR = 35.821, 95% CI = 4.591–279.490), who did not wear a mask while using pesticides (OR = 11.898, 95% CI = 1.061–133.440), and who did not wear boots while using pesticides (OR = 0.166, 95% CI = 0.031–0.890) had an increased likelihood of having a severe inhibition of erythrocyte AChE. This negative association could possibly protect those not wearing boots as boots might accumulate spilled pesticides (Table 5).

DISCUSSION

According to the pesticide handling findings, most of them had appropriate working behaviors, while a few of them had inappropriate practices, which led them to a higher risk of exposure. *Damalas* and *Koutroubas* [12] indicated that substantial exposure could also occur in or around the home due to storage of pesticide products and application equipment

Table 4. The association between self-reported symptoms and erythrocyte AChE (n = 71)

Self-reported symptoms	No	Yes	x^2	*P*-value
	n (%)	n (%)		
Respiratory system				
Dyspnea	50 (70.42)	21 (29.58)	1.65	0.19
Bronchorrhea	52 (73.24)	19 (26.76)	0.59	0.44
Running nose	46 (64.79)	25 (35.21)	0.13	0.71
Shortness of breath	51 (71.83)	20 (28.17)	4.53	0.03*
Cough	52 (73.24)	19 (26.76)	1.72	0.18
Gastrointestinal system				
Anorexia	64 (90.14)	7 (9.86)	0.85	0.35
Vomiting	62 (87.32)	9 (12.68)	1.94	0.16
Thirst	54 (76.06)	17 (23.94)	0.86	0.35
Stomachache	59 (83.10)	12 (16.90)	0.07	0.79
Urinary system				
Loss of urinary control	56 (78.87)	15 (21.13)	1.06	0.30
Glands				
Hypersalivation	44 (61.97)	27 (38.03)	0.33	0.57
Sweating	38 (53.52)	33 (46.48)	0.17	0.67
Eye system				
Red eyes	52 (73.24)	19 (26.76)	1.72	0.19
Blurred vision	38 (53.52)	33 (46.48)	0.01	0.95
Lacrimation	37 (52.11)	34 (47.89)	1.23	0.26
Irritation	32 (45.07)	39 (54.93)	2.94	0.04*
Skin system				
Skin rash/itching	36 (50.70)	35 (49.30)	0.01	0.91
Skin burning	67 (94.37)	4 (5.63)	1.53	0.22
Muscle system				
Numbness	37 (52.11)	34 (47.89)	0.40	0.53
Muscular twitching	58 (81.69)	13 (18.31)	1.32	0.25
Muscle weakness	53 (74.65)	18 (25.35)	0.37	0.54
Muscle fatigue	27 (38.03)	44 (61.97)	0.81	0.37
Central nervous system				
Headache	37 (52.11)	34 (47.89)	6.46	0.01*
Dizziness	40 (56.34)	31 (43.66)	8.24	0.00**
Sleep fragmentation	38 (53.52)	33 (46.48)	3.43	0.04*
Slurred speech	68 (95.77)	3 (4.23)	0.17	0.67
Ataxia	61 (85.92)	10 (14.08)	1.07	0.30
Confusion	55 (77.46)	16 (22.54)	1.60	0.21
Tremor	63 (88.73)	8 (11.27)	0.21	0.65
Irritability	50 (70.42)	21 (29.58)	0.59	0.44
Memory problem	25 (35.21)	46 (64.79)	5.58	0.01*

Note: *Chi*-square test, *p*-value = significant value (**p*-value < 0.05, and ***p*-value <0.01)

Table 5. Binary logistic regression analysis for determinants of the outcome variable (severe inhibition of erythrocyte AChE) (n = 71)

Independent variables	B	S.E.	Wald	*P*-value	OR (95% CI)
Age	−0.297	0.666	0.199	0.655	0.743 (0.201–2.742)
Alcohol consumption	−0.384	0.767	0.251	0.617	0.681 (0.152–3.061)
Smoking	0.186	0.760	0.060	0.806	1.205 (0.272–5.343)
Drinking while using pesticides (mixing, loading, and spraying)	3.579	1.048	11.655	0.001**	35.821 (4.591–279.490)
Smoking while using pesticides (mixing, loading, and spraying)	−0.360	1.405	0.066	0.798	0.698 (0.044–10.960)
Mask	2.476	1.233	4.031	0.045*	11.898 (1.061–133.440)
Gloves	0.014	0.711	0.001	0.984	1.015 (0.252–4.086)
Boots	−1.797	0.857	4.395	0.036*	0.166 (0.031–0.890)

Note: B = regression coefficient, S.E. = standard error, OR = odds ratio, CI = confidence interval, 0 = reference, erythrocyte AChE (0 ≥ 24.5U/g Hb, 1 < 24.5U/g Hb), age (0 ≤ 50 years, 1 > 50 years), alcohol drinking (0 = no, 1 = yes), smoking (0 = no, 1 = yes), smoking while using pesticides (mixing, loading, and spraying) (0 = no, 1 = yes), drinking while using pesticides (mixing, loading, and spraying) (0 = no, 1 = yes), masks (0 = no, 1 = yes), gloves (0 = no, 1 = yes), boots (0 = no, 1 = yes), *p*-value = significant value (**p*-value < 0.05 and ***p*-value < 0.01)

(sprayers) at home. However, farmers who were greatly aware of the harmful effects of pesticides were sometimes unable to recall this awareness in their practices [13]. A study of pesticide handling in Sudan's farmers reported that the greater part of them did not smoke, eat, or drink water during pesticide use, but all of them demonstrated poor practices of disposing of empty pesticide containers [14]. In addition, improper handling of pesticides contributes significantly to human health poisoning effects on various systems, such as the nervous, respiratory, muscular, digestive, hormone, reproductive, and lymphatic systems. Most of the participants utilized at least one PPE. Most farmers wore masks, long sleeve shirts, long pants, hats, shoes, and gloves, but a few of them wore protective eyewear and aprons, as reported in a previous study [15]. Although they used PPE to protect themselves from pesticide exposure, it was not sufficient for agrochemical resistance, except rubber gloves and boots. A previous study explained that farmers regularly used poor and nonstandard PPE due to a lack of knowledge, attitude, poor practice, and PPE availability [16].

The findings of this study demonstrated that the farmers' health was affected due to OP and CA pesticide intoxication during application. These conclusions are similar to those reported in a previous study [4]. Both pesticides can induce neurotoxic effects by inhibiting erythrocyte AChE activity. This study found an association between erythrocyte AChE changes and self-reported symptoms of shortness of breath, irritation, headache, dizziness, sleep fragmentation, and memory problems among pesticide applicators. This might be explained by the fact that the level of exposure integrated with the lack of appropriate use of PPE, such as protective eyewear, aprons, gloves, and shoes, was linked to symptoms of specific systems, such as eye irritation, skin rash or itching, and blurred vision. The high prevalence of these symptoms demonstrating chronic and neurotoxic effects could be associated with deficits in neurobehavioral performance and abnormalities in nerve functioning [17]. Farmers reported that there was a considerable decrease in the erythrocyte AChE activity showing severe inhibition (<24.5 U/g H), which was associated with shortness of breath, irritation, headache, dizziness, sleep fragmentation, and memory problems ($p<0.05$). At the same time, previous studies also indicated that the association between AChE inhibition and respiratory, eye, and central nervous systems decreased during high exposure [18]. The high prevalence of these symptoms revealed chronic neurotoxic effects associated with deficits in neurobehavioral performance and abnormalities in nerve functioning [17]. Even though there were statistically significant associations between the self-reported symptoms and erythrocyte AChE, it cannot be confirmed that the increased symptoms originated from occupational exposure to pesticides [4]. Self-reported symptoms of farmers might also be caused by mental stress, heat exposure, and heavy workload; thus, only levels of erythrocyte AChE activity cannot be used to confirm that these symptoms result from pesticide toxicity.

In addition, we examined risk factors associated with erythrocyte AChE as a biological marker of OP and CA pesticide exposure. The binary logistic regression analysis included variables with a *p*-value of <0.20 in the *Chi*-square test in the model, including age and alcohol consumption and smoking habits while using pesticides (mixing, loading, and spraying), masks, gloves, and boots. The finding showed a statistically significant association between the risk factors and erythrocyte AChE values in pesticide applicators, demonstrating that the risk of severe erythrocyte AChE inhibition (<24.5 U/g Hb) in farmers who drank while using pesticides (mixing, loading, and spraying) was 35.821-fold higher than in those who did not drink. In Thailand, they normally bring food from home to the paddy fields during the working period. Prior studies revealed that exposure of farmers to pesticides occurs primarily through drinking water and eating food contaminated when mixing and applying pesticides or working in paddy fields [19]. However, these risk behaviors, including drinking during pesticide handling, were not common in the farmers [4]. The risks of severe erythrocyte AChE inhibition (<24.5 U/g Hb) in farmers who did not wear masks and boots while using pesticides were 11.898-fold and 0.166-fold higher than in farmers who wore, respectively (not wearing boots as a good protection possibly due to spilled pesticide deposit). The severe erythrocyte AChE inhibition of pesticide exposure observed in this present study might be partly attributed to poor PPE used. This may be explained by the fact that a lack of PPE was found to increase the probability of lower erythrocyte AChE levels. As expected, farmers with less wearing of masks, gloves, and boots had more opportunities to contact the pesticides. The use of PPE was a significant aspect of individual prevention against exposure, except boots, because most OP and CA pesticides are highly lipid-soluble agents and are well absorbed through the skin [20].

CONCLUSION

The present study found that exposure to OP and CA pesticides occupationally was associated with erythrocyte AChE inhibition and self-reported symptoms among farmers. The risk factors were poor hygienic practices and inadequate use of PPE. When farmers did not use masks, gloves, and boots while applying pesticides but consumed food in the field, there was an increased chance of exposure to pesticides via ingestion, inhalation, and dermal contact. The best practice for farmers would be increasing awareness of pesticide handling by training regularly or using low-toxicity pesticides. In addition, farmers should avoid eating, drinking, and smoking during spraying to prevent pesticide exposure. The pesticide exposure in the study area is raising public health concerns for the health of farmers. Therefore, government regulations about working practices and hygiene among farmers, such as controlling all aspects of pesticide storage and disposal, appropriate application, equipment cleaning, and proper use of PPE, are critically needed.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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