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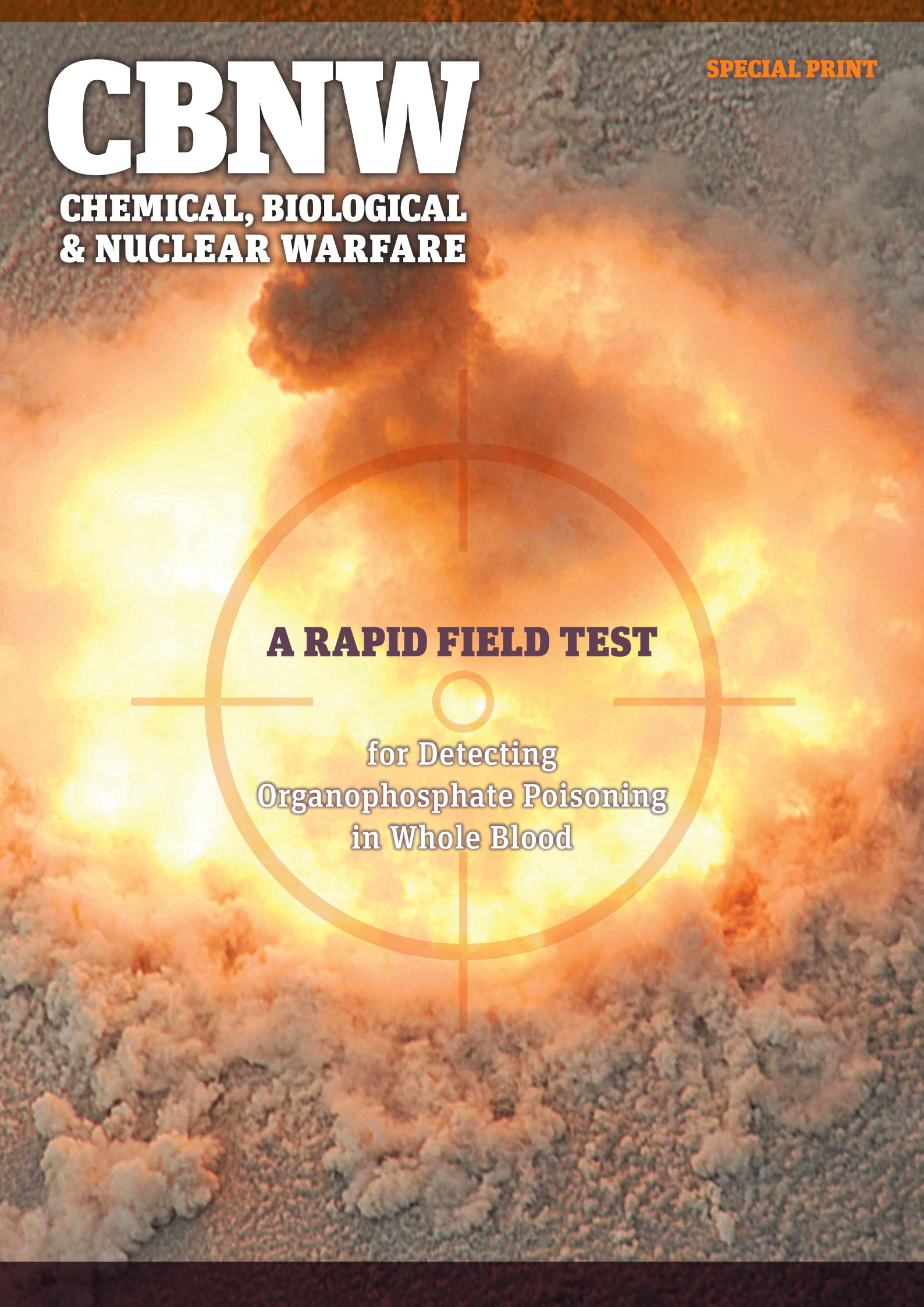


A RAPID FIELD TEST

for Detecting
Organophosphate Poisoning
in Whole Blood

NERVE AGENTS – limit the consequences

Dr Sebastian Klaus and Marion Nies describe a rapid field test for detecting organophosphate poisoning in whole blood

The newly available CE certified cholinesterase (ChE) IVD device, ChE check mobile, provides an important diagnostic capability that allows rapid and accurate determination of acetyl cholinesterase (AChE) and butyryl cholinesterase (BChE) activity in whole blood. The device can be used in both permanent medical treatment facilities and under field conditions, in particular during deployed field operations when exposure to organophosphates (OPs) such as nerve agents is suspected.
Even in cases of weak or unspecific symptoms, the ChE check mobile is small and easy to use, and enables medical officers in charge to establish rapid and accurate diagnosis and to provide timely and adequate antidote therapy. The practical test device allows precise and reproducible determination of AChE and BChE activity within four minutes at ambient temperatures of 10°C to 50°C. The simple test procedure requires only a few menu-driven operations and the interpretation of test results is supported by supplemental information and instruction; thus, enabling the test execution after short training.

FIGURE 1: Components of the ChE check mobile

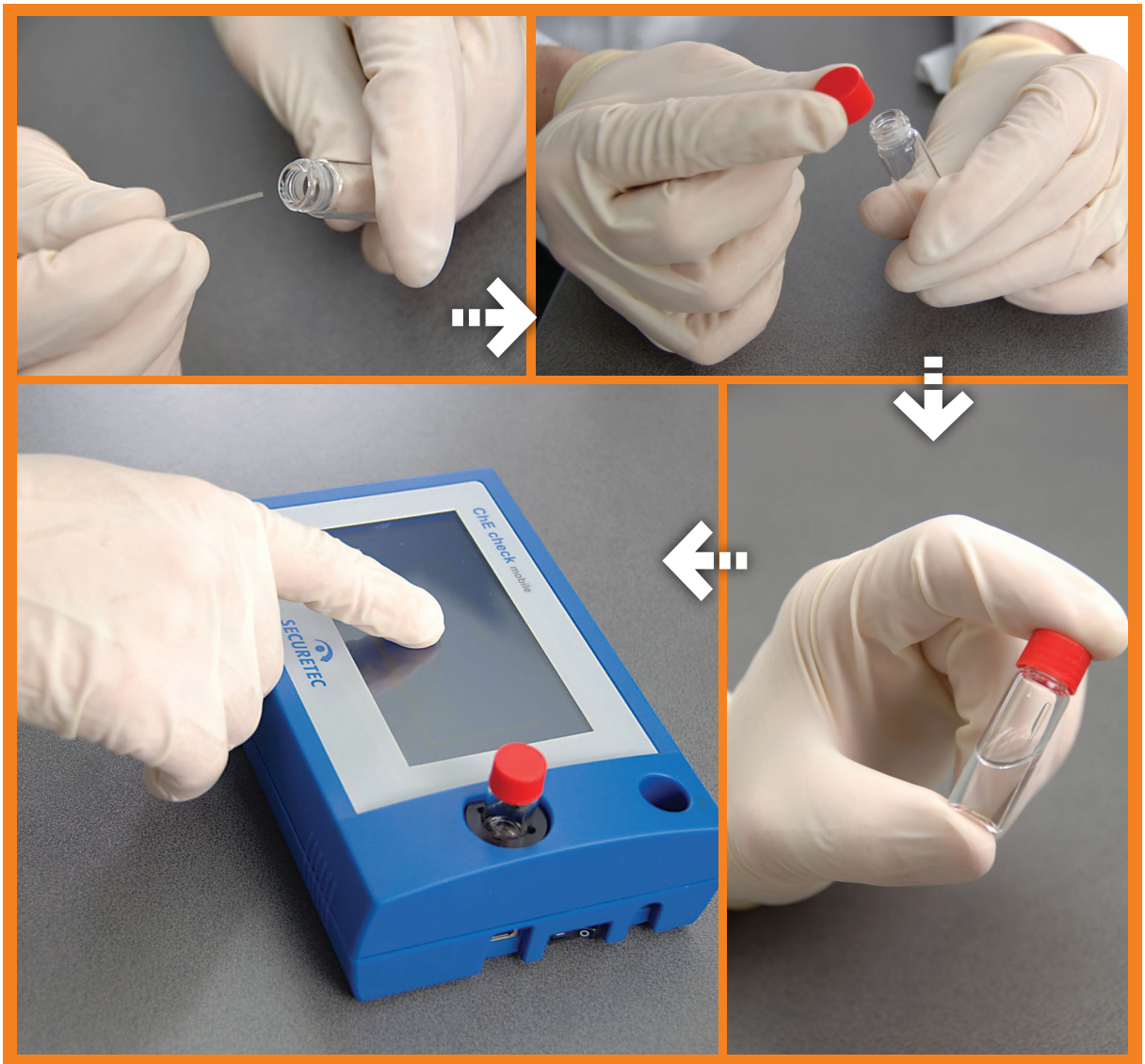


FIGURE 2: Steps for AChE determination using the ChE check mobile. Addition of blood sample (upper left), positioning of the reagent cup (upper right), mixing of the solution (lower right) and initiation of measurement (lower left).

Effects of OP

Irreversible inhibition of the pivotal enzyme acetyl cholinesterase is the predominant toxic effect of many organophosphorus compounds and carbamates (organic compounds derived from carbamic acid). Many OPs and carbamates are still being used worldwide as agricultural pesticides, while some have been incorporated into and abused as CWAs (chemical warfare agents).

Inhibition of AChE by OP results in accumulation of acetylcholine in the synaptic cleft (the microscopic gap between neurons) of cholinergic-innervated tissues, making many body organs dysfunctional and ultimately resulting in death by central and peripheral respiratory failure.

Need for a rapid test

Until now, evaluation of clinical symptoms has been the only possibility for on-site diagnosis of OP poisoning because there were no field devices available for rapid and precise on-site determination. Particularly in cases when symptoms were weak or unspecific, a clinical laboratory confirmation using complex, expensive and time-consuming analytical methods such as GC-MS (gas chromatography/mass spectrometry) became inevitable. As a result, lack of prompt diagnosis did not allow a timely and adequate antidote therapy. For this reason, ChE check mobile was developed by Securetec Detektions-Systeme AG (Brunnthal, Germany) in close co-operation with the Bundeswehr Institute of Pharmacology and Toxicology (Munich, Germany).

Method and device

The determination of AChE and BChE in whole blood samples is based on the well-known Ellmann method used since 1961. The Ellman reagent forms a yellow colour which is measured by a photometer. The rate of colour formation is proportional to the amount of either AChE or BChE.

Check Mobile consists of a small photometer (18 x 12 x 5 cm) and two different reagent kits (Fig. 1) and is designed for the determination of exposures to AChE and BChE inhibitors. The photometer contains a large touch-screen display and is used for the measurement of haemoglobin concentration and the enzymatic AChE/BChE reaction products.

Power supply is provided via rechargeable battery or power cable; data from measurements can be internally stored and transferred via a USB interface. There are two different reagent kits, one each for determination of AChE or BChE activity. Each kit contains all necessary items such as sample cuvettes, reagent caps for AChE or BChE determination and capillaries for blood sampling.

Result in 4 minutes

ChE check mobile allows reliable, accurate and reproducible determination of AChE or BChE activity under field conditions within four minutes from sampling until test results are displayed. Test execution is carried out by using the following menu-driven steps indicated on the touch screen (Fig 2 on previous page):

1. Baseline measurement (blank value) by positioning of the sample cuvettes into the analysis unit
2. Addition of 10 µl (microlitres) blood sample by using a capillary, shaking the sample cuvettes and measuring the hemoglobin content
3. Addition of reagents by positioning the desired, colour-coded lid onto the cuvettes (AChE or BChE) and mixing
4. Photometric determination of AChE or BChE activity
5. Display of results of AChE or BChE activity (normalized to the haemoglobin content of the sample) including result interpretation and further instructions.

Results for validation

ChE check mobile was classified as IVD (in vitro diagnostic) and a Class I Medical Device according to EU regulations. In order to allow CE labeling, the device had to pass extensive validation testing.

Enzymes such as AChE and BChE show temperature-dependent activity. Therefore, an important prerequisite to assure ChE check mobile will perform well under field conditions is its ability to function in a wide range of temperatures and to normalize values to 37°C. Accordingly, the device contains a temperature sensor to determine the actual assay temperature and a mathematical algorithm to normalize temperature effects.

Fig. 3 shows examples of AChE activities determined in native and OP inhibited samples at different temperatures showing that determination of AChE and BChE activity at temperatures from 10°C to 50°C is possible.

In order to use ChE check mobile for laboratory diagnosis of exposure to AChE/BChE inhibitors, the

Parameter	Coefficient of determination (R^2)
AChE (n = 10)	0.92
BChE (n = 10)	0.98

Table 1. Correlation of results of AChE and BChE activity determination in whole blood samples, using ChE check mobile and a reference method.

Parameter	Coefficient of variation (CV) (%)	
	AChE	BChE
Intra-Day variability (n = 91)	1.3	▸ 4.5
Inter-Day variability (n = 45; 5 days)	0.8	▸ 2.5
Inter-Operator variability (n = 30) by measurement of 10 samples by 3 persons)	0.7	▸ 4.2

Table 2. Reproducibility of AChE and BChE activity determination in whole blood samples using ChE check mobile.

device needs to provide precise and accurate data on AChE and BChE activity over a wide range. Determination of AChE and BChE activity in whole blood samples with very different enzyme activities in fact confirmed a linear correlation in a range from minimal to above-average physiological values, as shown in Fig. 4.

Another part of the validation was the comparison of ChE check mobile with a reference method – a modified Ellmann method established at the Bundeswehr Institute of Pharmacology and Toxicology and accredited under DIN EN ISO 15189. Determination of variations of AChE and BChE activities with both methods demonstrated correlations that, with regard to biological samples, can be considered excellent (correlation of both methods: $R^2 > 0.9$; Table 1). In addition, the reproducibility of the device (intra-day variability, inter-day variability and inter-operator variability) was shown to be below 5% (Table 2).

Further measurements were done using real samples from OP pesticide-poisoned patients. As shown in Fig. 5, a good correlation between the ChE check mobile and the reference laboratory method is evident, proving the functionality of the device for field applications.

FIGURE 3: Determination of ruggedness of ChE rapid test IVD results against temperature effects; measurement of native and inhibited AChE activities in whole blood samples (n=4, means ± SD).

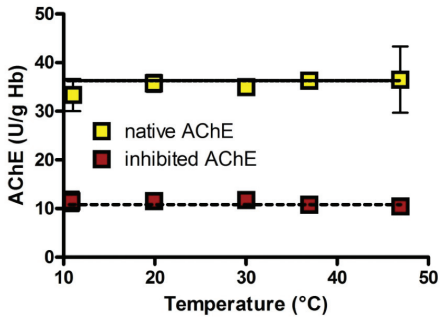


FIGURE 4: Linear correlation of AChE values determined by ChE rapid test IVD in blood samples with different (known) AChE activities (n = 4, Means ± SD).

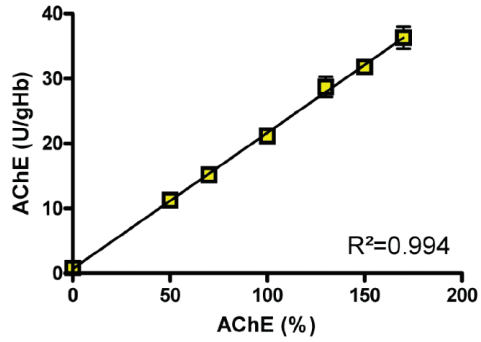
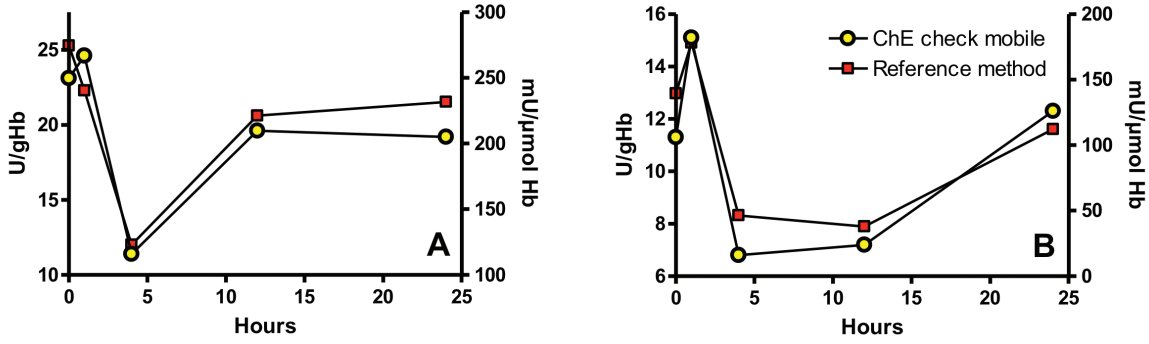


FIGURE 5



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