MEASURING DEVICES AND METHODS FOR DETERMINING BLOOD ALCOHOL CONCENTRATION

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PREFACE

Investigations into various aspects of alcohol consumption in traffic are included in the working programma of the Institute for Road Safety Research SWOV by order of the Ministry of Transport and Waterways and for account of the Ministry of Social Security and Public Health. Within this scope a report was published in 1968 (2nd edition, 1969) entitled:
Alcohol and Road Safety - Countermeasures and research; A critical survey of the literature.

As a sequel to this report attention was given to the way in which it is possible to measure the quantity of alcohol in the human body. This resulted in a study of the existing literature and an additional investigation. The outcome is given in the following documentation which must be seen as a supplement to the brief paper read by P.C. Noordzij, research psychologist, at the 5th International conference on alcohol and traffic safety, held in Freiburg in September 1969. The paper will be included in the proceedings of this conference. It is, however, not necessary to have the text of the paper when reading this documentation.

An appraisal of the various methods for measuring an amount of alcohol in the human body is necessary to arrive at a scientific investigation of the effect that alcohol has on driving ability. When applying methods for forensic purposes, some aspects that have been left out of consideration here, would be of interest, for instance the possibility of a counter-examination and the inevitable lapse of time between the stopping of the drivers suspected of infringement of Section 26 Netherlands Road Traffic Act and the moment when the blood or breath samples are taken.

An investigation of these aspects was not included in the assignment. It would, however, enhance the value of the investigation if these aspects were also considered should a more extensive research be contemplated.

It will be hardly necessary to have specialist knowledge to read the literature survey. This also goes for the description of investigations. However, to understand the interpretation of the investigation results, some knowledge of statistical methods is essential. Even without this knowledge, the conclusion can be drawn that more extensive research is required.

This documentation has been compiled by P.C. Noordzij, research psychologist (Human Factor Department SWOV) aided by: Analytical Centre of the Central Laboratory TNO-Delft (Dr. G.J. van Kolmeschate, director; L.J. Hoogendoorn; P. Slingerland); Central Bureau for Medical Hygienic Examinations, The Hague; Central Laboratory for Clinical Chemistry of the Dijkzigt Hospital, Rotterdam (Mrs.Dr. H.J. Leijnse-Ybema, head Biochemical Laboratory); Municipal police Middelburg (Dr. D. van Ooijen, superintendent of police); Municipal police Rotterdam (H.Th.P. Cremers, police-surgeon); M.J. Koornstra, statistical adviser SWOV; Institute of Medical Physics TNO, Utrecht (Ir. J. Kuiper); Lucien Etzlinger, Appareils Electroniques, Genève; N.V. Philips, Eindhoven.

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SUMMARY

A review of present measuring devices and procedures, based on information obtained from the literature, has led to the following conclusions:

The selection of a method of determining the b.a.c. for research purposes amounts to a choice between blood and breath analysis. Further research is required before this choice can be made.

The SWOV has contributed to this by means of laboratory experiments in order to assess the calibration and accuracy of several instruments. In addition, preliminary technological investigations have been made preparatory to more extensive research into the value of blood and breath analysis under practical conditions. This more extensive research has, however, been delayed by unforeseen circumstances outside the scientific sphere.

NETHERLANDS ROAD TRAFFIC ACT

Section 26

1. It is forbidden for the driver of a motor vehicle, a bicycle or any other carriage or vehicle to drive therewith on a road while under the influence of the consumption of alcoholic drink to such an extent that he cannot be considered to be capable of properly driving the said motor vehicle, bicycle or other carriage or vehicle.

2. It is forbidden for the driver of a motor vehicle to drive the said vehicle on a road while the person who, in accordance with the conditions referred to in paragraph 3(e) of Section 1, is deemed to be driving the vehicle under his direct supervision, is under the influence of the consumption of alcoholic drink to such an extent that he cannot be considered to be capable of properly driving the said vehicle.

3. For the purposes of the present Section any substance, the consumption of which the driver known or may reasonable be assumed to know can impair his ability to drive, is assimilated to alcoholic drink.

1. INTRODUCTORY

Alcohol consumption influences driving ability. This is an undoubted fact.

Questions only partly solved, however, are:
(a) how much alcohol can be consumed before there is any question of diminished driving ability?
(b) what psycho-physiological functions of importance to driving are influenced by alcohol?
(c) what is the frequency of "non-sober driving"?
(d) what biographic factors are connected with this?
(e) what is the effect of countermeasures to limit "non-sober driving"?

The Institute for Road Safety Research SWOV has an extensive research programme in order to find answers to these questions.
For each such research project it is necessary to know the exact amount of alcohol in the human body. Alcohol consumed is carried by the bloodstream to various parts of the body. It is customary to express the quantity of alcohol in the blood as the blood alcohol concentration (b.a.c.), in grammes alcohol per litre blood (as a promille: °/oo).

The above-mentioned research projects depend upon an accurate but rapid and simple method of determining the b.a.c., if possible without medical assistence.

2. REVIEW OF PRESENT DEVICES AND METHODS

It is not intended to deal exhaustively with all existing methods; for these reference is made, among others, to Grüner (1967) and A.M.A. (1968), while a less detailed discussion can be found in Goldberg & Havard (1968).
The following review serves merely as a background in considering the importance of SWOV's investigations presented in this report.

Methods of determining the b.a.c. can be divided into direct and indirect methods.

The direct methods are those in which a sample of blood is analysed. The indirect methods are those in which, for example, a sample of urine, breath or saliva is analysed, followed by an estimate of the blood alcohol concentration.

2.1. Blood analysis

Analysis of a sample of blood presents the best prospects of exact determination of the b.a.c. at the time of sampling.
There are however a number of sources of error liable to influence the results.

2.1.1. Sampling

Origin of the blood

Depending on the part of the body from which blood is taken for

analysis, determination of the b.a.c. may produce different results (Harger, 1962).
This is because the transport of alcohol from the gastro-intestinal tract to other parts of the body via the bloods tream takes some time. Differences in the alcohol concentration of blood from various parts of the body therefore occur shortly after consumption of alcohol (the absorption stage). The differences vanish immediately this stage has been passed and when the decomposition of alcohol preponderates (the elimination stage).
It is reasonable to assume that the alcohol carried via the blood to the brain has the most important effect upon driving ability.

Hence the stated b.a.c. should be the closest possible approximation of the alcohol content of this kind of blood. The blood going to the brain is arterial blood.
It is difficult to obtain a sample of this from living organisms. A sample of capillary blood whose composition approximates that of arterial blood would therefore have to suffice. In practice, however, taking a sample of capillary blood proves to cause many problems (Payne et al., 1967; Payne, 1968).
An adequate quantity of venous blood is obtainable by simple medical means. But this must always be done under the responsibility of a medical doctor.
When venous blood is used, one must however realise that in circumstances when one does not know whether the person concerned is in the absorption or the elimination stage, there is a risk of the b.a.c. then found differing from the arterial b.a.c. which is, in fact, what one is interested in.

Fluctuations in b.a.c.

There has been little investigation of the fact that the b.a.c.'s of samples taken from one and the same individual at brief intervals are found to fluctuate. These fluctuations are believed not to be due to inaccurate analysis, but to indicate actual fluctuations in the blood composition occuring after the recent consumption of alcohol (Ponsold, 1965). For this reason b.a.c. values would be desirable giving

averages for short periods of time. This is difficult to achieve even under laboratory conditions (Hinckers, Jeschonnek & Knüpling, 1968; Hinckers, 1968). The b.a.c. found for a single sample therefore differs from the required b.a.c. value.

2.1.2. Storage of blood samples

It is a known fact that the alcohol concentration changes if samples of blood are not carefully stored.
Factors of importance include the temperature (Grüner, 1967), coagulation, evaporation, absorption through containers (Payne, 1968). The question is whether the error is really of practical importance in using a particular procedure. In view of the findings of Begg, Hill & Nickolls (1962) and Enticknap & Wright (1965), this question must be answered in the affirmative. These workers divided the blood they took into two samples that received equal treatment. The difference between the results for the two samples was distinctly greater than the difference between two determinations of one sample. Apparently the use of small polypropylene cups containing an anti-coagulant, however, greatly reduces the effect of coagulation, evaporation and absorption (Payne, 1968). It is still questionable whether it is worth going to the trouble of cooling samples immediately.

2.1.3. Analysis method

A large number of methods of blood analysis are known, all of which have their advantages and disadvantages.
On the whole the gas chromatography method is regarded as the most valuable (Froentjes, 1968; Goldberg & Havard, 1968). This method is very specific (i.e. shows a minimum influence of substances in the blood besides the alcohol consumed) and very accurate (i.e. repeated determinations show only slight differences between the values).
The quantity of blood required is small, and analysis takes little time. A drawback is the high cost of the gas chromatography equipment. But this is offset because it can also be used to analyse blood for substances other than alcohol. Easy-to-operate portable gas chromatography sets recently appeared on the market.

Other widely applied procedures are the chemical methods (the Widmark method being one of the best known) and the enzymatic methods (especially the A.D.H. method).
Specificity and accuracy of these methods, however, are lower than those of gas chromatography.
The accuracy of gas chromatography and the enzymatic methods apparently declines at higher b.a.c. values (Grüner, 1967). As to the chemical methods, however, there are indications of increasing accuracy with higher b.a.c.'s (Grüner, 1967).

The accuracy of blood analysis in fact depends not only on the physical and chemical principles of the analysis method, but also differs from laboratory to laboratory (Grüner, 1967; Fox et al., 1965).
Besides this, the accuracy of routine determinations will probably be lower than that calculated for an analysis method using standard samples (Grüner, 1967).

2.1.4. Conclusions

The ultimate error of the method as a whole consists of a difference in analysis of a sample of blood as compared with the average "true" alcohol content of arterial blood over a brief period of time.
This error, which arises from defective sampling, storage and analysis, may thus be significantly greater than the error in the method of analysis itself. And yet only the latter error is generally mentioned.
In theory, enough is known about the other errors, but too little quantitative information exists about their practical significance.
This information is necessary in order to find out what aspects of the method can be improved. Quantitative information on the method as a whole is needed for fair comparison with alternative methods, especially the indirect method.

2.2. Breath analysis

As stated above, blood analysis provides the best prospects of accurate b.a.c. determination at the time of sampling. But taking a blood sample is a medical process. The indirect methods require no medical

action. The estimate that has to be made in the case of urine and saliva analysis is so inaccurate, however, that these methods are unsuitable as alternatives for blood analysis if anything like accurate determination of the b.a.c. is required. Detailed discussion of urine analysis is contained in Froentjes (1962), Kaye & Cardona (1965) and Payne (1967). For saliva analysis see Grüner (1967) and Gostomzyk, Liebhardt & Henn (1968).

It will be shown that breath analysis is an exception to this. If breath is blown properly, the alcohol in the breath comes from blood flowing through the lung artery. Even during the absorption stage the b.a.c. of this blood closely resembles the b.a.c. of blood flowing ot the brain. Moreover, alcohol in the breath is "collected" for the duration of one breath, and the alcohol content of breath can thus be regarded as an average value for a brief period of time (Ponsold, 1965).
Besides this, breath analysis can be carried out on the spot without the drawbacks of storing and transporting samples.
Harger (1962) saw sufficient grounds for favouring an estimate of the arterial b.a.c. based on breath analysis in preference to blood analysis.

2.2.1. Tubes and balloons

The most widely known breath analysis devices are the "tubes" or "balloons". The tubes are filled with a chemical (usually silica gel crystals impregnated with potassium bichromate and sulphuric acid) with changes colour upon contact with alcohol. The balloons are used to indicate the amount of air blown in.
B.a.c. determination with such tubes is of doubtful accuracy. Most versions indicate whether the b.a.c. is above or below a given limit. Investigations regarding the value of these tubes often overlook that the number of correct determinations with them depends on the distribution of the "true" b.a.c.'s in the sample investigated. If the "true" b.a.c.'s are well away from the limit indicated by the tube, practically all the determinations will be right. If, however, the "true" b.a.c.'s are close around this limit, even a very reliable method will still produce a large number of faulty determinations. It

is thus important in choosing a b.a.c. determination procedure to know what the distribution of "true" b.a.c.'s in the group of persons to be examined will be.
Some tubes offer more differentiated quantitative b.a.c.'s.

2.2.2. More elaborate devices

The best known, more elaborate device was designed by Borkenstein and called the "breathalyzer". The "ethanographe" manufactured in Switzerland works on the same principles and differs in detail only from the "breathalyzer". Kitagawa and Wright developed the "Hermes" device. These are the three best known ones. With these three devices breath analysis is based on the same chemical reaction as with the tubes. In their construction, however, an attempt has been made to achieve greater accuracy in a number of respects than with the tubes. These are:
(a) the volume of air analysed
(b) the origin of the exhaled breath (from the depths of the lungs)
(c) temperature of chemical reaction
(d) quantification of the chemical change.

2.2.3. Sources of error

A number of sources of error continue to exist even with these devices. These are discussed below.

2.2.3.1. Accuracy of the device

The accuracy of the device can be determined by analysing moist air/alcohol mixtures with known alcohol concentrations (simulated breath) with the devices.
For the "breathalyzer" this has been done by Britt & Borkenstein (1965), Franklin & Sutherland (1965) and others. Accuracy is apparently independent of the b.a.c. level and is of the same order as, for instance, the chemical blood analysis method. By means of a number of improvements, Fox, Lower & Fox (1962) were able to make the "breathalyzer" much more accurate still.
The "Hermes" was investigated by Entick nap & Wright (1965). The accuracy

of this device was found to decrease with higher b.a.c.'s. At low b.a.c.'s it was as accurate as the "breathalyzer".

2.2.3.2. Use

As a rule, the more complicated the device and the more difficult it is to use, the easier it is to make errors. Although this, of course, applies to the breath analysis devices as well, their use by many police forces, including those in America, Australia and Switzerland, indicates that problems in this respect can be eliminated by training the users.

2.2.3.3. Way of blowing

The influence of the way of blowing by individuals as an additional source of error can be investigated by having a group of persons, under the influence of alcohol in varying degrees, blow into the device a number of times in succession. Begg, Hill & Nickolls (1962; 1964) did this with the "breathalyzer" and the "Hermes" and found lower accuracy than obtained by other investigators with simulated breath. These writers state that even then the accuracy of the devices remains independent of the b.a.c. level.

The variation occuring through blowing can be limited in different ways. The simplest is to instruct the persons to blow out as far as possible. It is difficult for the user of the device, however, to determine whether these instructions are followed.
This procedure is likely to be most accurate with co-operative subjects. For less co-operative ones, however, there will have to be a method with more scope for supervision.
Coldwell & Grant (1963) determined the accuracy of the "breathalyzer" when used by the police for persons suspected of "non-sober driving". Its accuracy was lower than found by Begg, Hill & Nickolls (1964). This may, however, be due to non-expert use (by police officers), or to unwillingness on the part of the subjects, or to the time elapsing between first and second analysis (10 to 30 minutes) in which the "true" b.a.c. may have changed considerably.
According to Wright (1962) the variation in blowing is caused primarily by the temperature of the mouth. By keeping the mouth closed for five

minutes before exhaling the breath for analysis, this variation is practically eliminated.
Harger uses a procedure in which the persons breath a specified quantity of air in and out several times in succession (Harger, 1962; Grüner, 1967).
Monnier (1965) and Coldwell & Grant (1963) demonstrate that after eight seconds' blowing, the last 57 cc exhaled air is almost certainly alveolar air.

Blowing with the "breathalyzer" and the "ethanographe" is checked as follows. The breath passes from below into a vertical cylinder closed at the top with a piston. A red lamp shows that the piston is in its lowest position (the cylinder is then empty), a green lamp shows its highest position (there is then 57 cc of breath in the cylinder). In this way it can be checked whether the person has breathed out at least 57 cc air. The "Hermes" allows the first 500 cc of air to escape and then collects another 100 cc. A lamp indicates when this has happened. According to Enticknap & Wright (1965) the alcohol content of breath so obtained is 90% of that of alveolar air. This procedure is also claimed to be usable for children and for adults with respiratory defects.

Efforts have been made with other devices (Harger's "drunkometer", Forrester's "intoximeter") to avoid the variation in blowing by means of simultaneous determination of the CO_2 and alcohol content of samples of breath. Based on this CO_2 value, the alcohol content of the breath sample was then converted to the alcohol concentration of alveolar breath.

$$\frac{CO_2 \text{ (alveolar)}}{CO_2 \text{ (sample)}} \times \text{(sample) alcohol} = \text{alveolar alcohol}$$

This procedure - which means complicating breath analysis still further - did not give the desired results (Dubowski, 1962; Grosskopf, 1962; Grüner, 1967).

It is essential to find out which procedure is least sensitive to the way of blowing by cooperative and non-cooperative persons before the accuracy of breath analysis can be definitely decided and the optimum conversion of breath to blood alcohol concentration can be achieved.

2.2.3.4. Alcohol remaining in the mouth

Breath analysis may be influenced by alcohol still in the mouth. The analysis would then give too high a result. Experiments have shown that 20 to 30 minutes after consumption of alcohol in the influence of alcohol left in the mouth is no longer detectable in breath analysis (Monnier & Ruedi, undated; Coldwell & Grant, 1963; Gostomzyk, Liebhardt & Henn, 1968). When tests are made without knowing when alcohol was consumed by the persons being tested, another analysis can be made about 20 minutes later with persons who are shown to have consumed alcohol, in order to eliminate the possibility of alcohol remaining in the mouth affecting the result. Rinsing the mouth with water before a sample of breath is taken also reduces the risk of it containing alcohol that has remained in the mouth after consumption.

2.2.3.5. Eructations

Belching by persons with alcohol in the stomach can temporarily increase the alcohol content of breath in the mouth. Such cases can be detected by repeating the analysis.

2.2.3.6. Condensation and saliva in the device

If there were moisture in the device in the form of condensed vapour or saliva, for instance, this would absorb part of the alcohol from the breath mixture blown in. This would make the result too low. Saliva can be kept out by using a mouthpiece with a filter, and proper warming of the device will prevent condensation of water vapour.

2.2.3.7. Conversion of breath to blood alcohol concentration

The breath analysis device determines the alcohol content of the breath; the analysis result shown by the device, however, is converted to b.a.c. For this purpose it is assumed that the ratio between breath alcohol concentration and (arterial) blood alcohol centration is 1 : 2100 at 34° C (the assumed temperature of exhaled air). In actual

fact, this ratio varies from individual and from time to time (Entick-nap & Wright, 1965). An important factor in this respect is the way in which the breath sample has been obtained i.e. the way of blowing of the subject.
The ratio at which this variation is at a minimum and the amount of this minimum variation can be investigated by analysing the breath of a large group of persons simultaneously and determining the b.a.c. as exactly as possible.

Data given by Fox et al. (1965) indicate that the ratio for the "breathalyzer" is around 1 : 2100 when co-operative persons are instructed to breath out as deeply as possible.
For the "Hermes", Entícknap & Wright (1965) show that for each of two persons tested the ratios are smaller than 1 : 2100.
These investigations also demonstrate that the original calibration by the manufacturer is not alway in accordance with the stated ratio of 1 : 2100.
If a ratio is taken for which the variation is at a minimum, deviations between results of breath and blood analysis are hardly greater, if at all, than the differences between two successive breath analyses for one and the same person (Fox et al., 1965; Lereboullet et al., 1961).

2.2.3.8. Specificity
A drawback that is sometimes raised to breath analysis is that the method is not specific for alcohol. The chemical reactions on which the analysis is based however are identical to those of chemical blood analysis methods (including the Widmark method).
This objection therefore applies equally to the latter methods, which in fact are rightly regarded as acceptable.

2.2.4. Recent developments

At the Salon des Inventeurs in Brussels (March 1969) an automatic and electronic "ethanographe" was exhibited. American firms advertise portable units based on gas chromatography and infra-red spectrography. According to their makers, these devices put an end to all problems of accuracy, operation and specificity. It is therefore expected that

these devices will soon be tested (or have already been tested) by independent researchers.

2.2.5. Conclusions

The accuracy of analysis of moist air/alcohol mixtures with the "breathalyzer", "ethanographe" and "Hermes" is definitely acceptable in comparison with chemical blood analysis procedures. Tests made in which the breath and blood of co-operative subjects were examined simultaneously under laboratory conditions likewise give good results. But not enough is known about the influence of the way of blowing both by co-operative and non-co-operative persons under realistic conditions as regards:

(a) reproducibility of breath analysis
(b) ratio between breath alcohol concentration and blood alcohol concentration
(c) variations in this ratio in a group of persons.

2.3. Summary of Section 2

The review shows that the choice of a method of b.a.c. determination amounts to a choice between blood analysis and breath analysis procedures. In order to make this choice, however, information must still be obtained on:

(a) the accuracy of the blood analysis method as a whole
(b) the accuracy with which the b.a.c. ca be predicted from analysis of a sample of breath and the way in which the breath analysis device should be calibrated for this purpose
(c) aspects of both methods requiring improvement.

The next part of this report will describe how SWOV has assisted (and hopes to go on assisting) with the collection of this information.

3. RESEARCH

Section 2 pointed out the need for research into blood and breath analysis methods under practical conditions. From considerations of efficiency this research was incorporated in investigations into questions (c), (d) and (e) (See Section 1).

To be certain that all data which the combined investigations had to supply could in fact be recorded, preliminary technological investigations were made in Middelburg in mid-1968.
The blood and breath analysers were first tested in the laboratory.

The co-operation of the police (inter alia by stopping drivers) was an essential condition for these preliminary investigations.
It was agreed that drivers who were stopped and whom the police believed to be liable for prosecution under Section 26 of the Netherlands Road Traffic Act (see p. 8) would not be involved in the enquiries. While the preliminary investigations were being carried out it was found that - notwithstanding the arrangements that had been made - legal drawbacks had been raised about the co-operation of police officers. As the problem could not be cleared up in time, the results of these preliminary investigations will have to suffice for the purpose of this report.

In view of the arrangements that had been made, drivers with relatively high b.a.c.'s were expected to be excluded. But in order that blood and breath analyses could be compared in these cases as well, it was planned to obtain the assistance of a police surgeon. This plan likewise had to be abandoned for reasons of legal policy.

Firstly, therefore, the laboratory experiments will be discussed and then the preliminary technological investigations.

3.1. Laboratory experiments: Blood analysis

All blood analyses were made at the Central Laboratory for Clinical Chemistry of Dijkzigt Hospital, Rotterdam, which obtained a Pye Type 104 gas chromatograph for this purpose. A brief description of the measurement procedure is given in Appendix 1 (see p. 49).

3.1.1. Calibration

Figure 1 (see p. 23) shows the results of a series of measurements for calibrating the device. In calibration, standard ethanol [1] solutions were diluted with a standard propanol solution and analysed. The result of measurement is given as an ethanol/propanol ratio, from which the alcohol content must be derived. As the figure shows, the relationship between the two variables is approximately linear.

In practice, preference will be given to a calibration curve running through the origin of the graph.
Statistically, the linear regression curve for predicting the ethanol concentration form the ethanol/propanol ratio gives the greatest accuracy. This curve does not necessarily pass through the origin of the graph.

In using the regression model, [2] it must be assumed that the extent of the error in predicting the ethanol concentration from the ethanol/propanol ratio is independent of the alcohol level.
We checked the assumption statistically as follows. The ethanol concentration was predicted with the aid of the regression formula for its prediction from the ethanol/propanol ratio.

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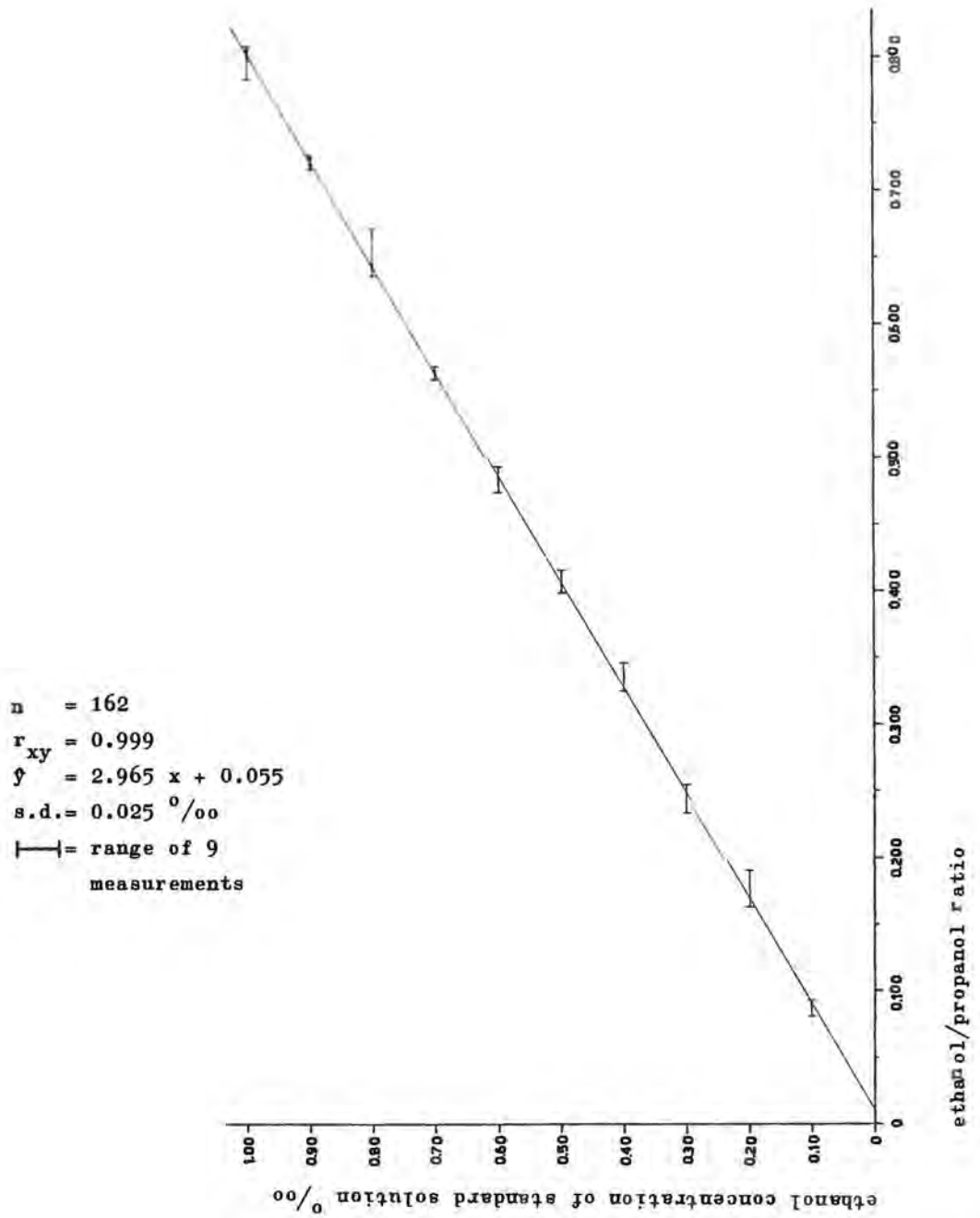
1) Ethanol is the chemical name for consumable alcohol.

2) A full discussion of the linear regression model can be found, for instance in Hays (1963), Gulliksen (1950), Ezekiel & Fox (1966).

Figure 1. The relation between ethanol concentration of a standard solution and ethanol/propanol ratio as int. st. propanol 0.25 °/oo



The difference between predicted and actual concentrations was squared (d^2) and correlated with the actual concentration (y). Whether the extent of the error of estimation depends on the alcohol level must be shown by a significant linear correlation coefficient (r^2_{dy}). This r, calculated for the subject matter of Figure 1, is 0.057 (non-significant) *).

3.1.2. Accuracy

The difference between predicted and actual ethanol concentrations can thus be expressed as a standard devation (s.d.) applicable to the entire range of ethanol concentrations measured. This s.d. can be calculated with the formula:

$$s.d. = s_y \sqrt{1 - r_{xy}^2} \qquad (1)$$

in which:

x is the ethanol/propanol ratio

y is the ethanol concentration

s_y is the standard deviation of y values for the samples examined

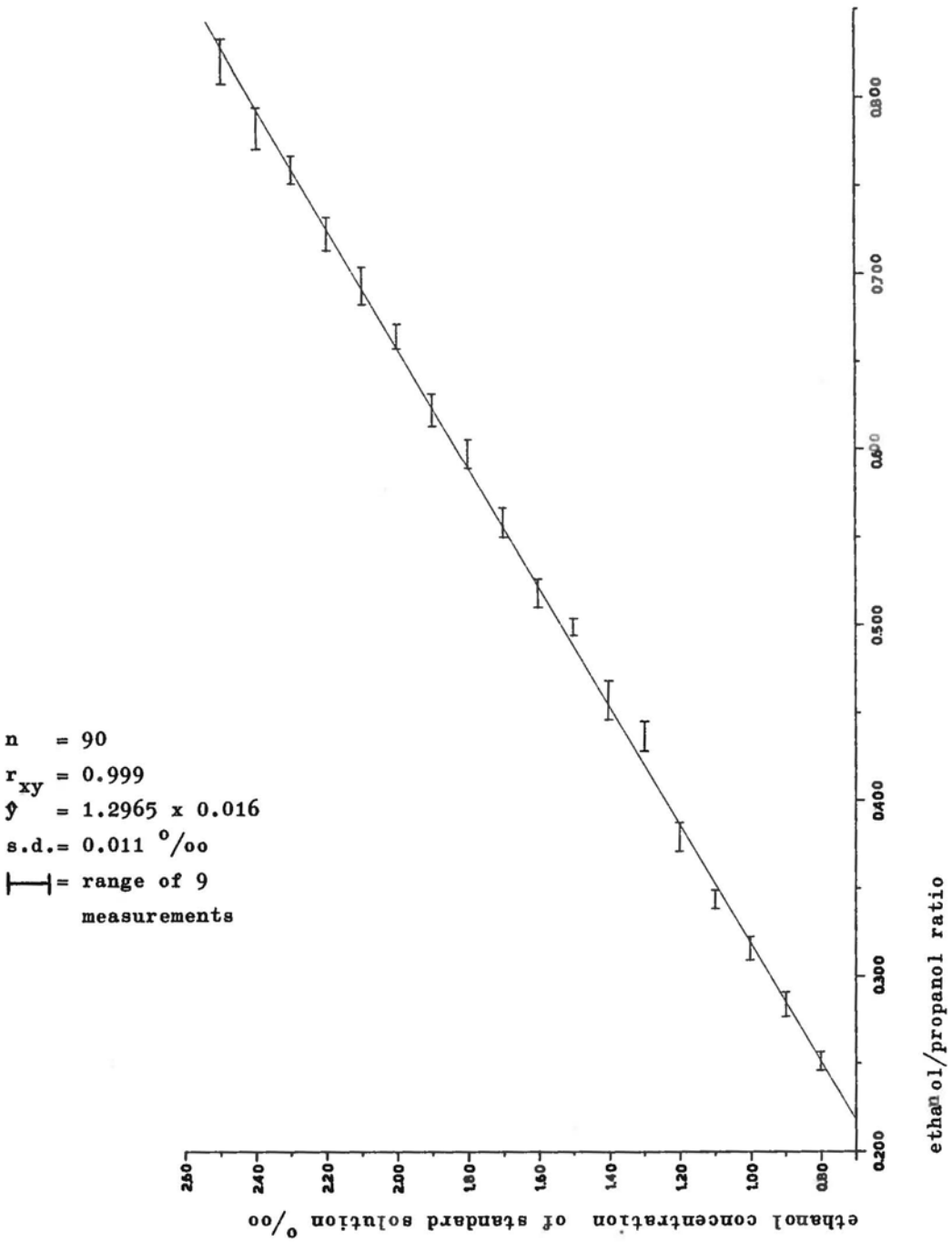
r_{xy} is the linear correlation coefficient between x and y.

For the subject matter of Figure 1, s.d. = 0.011 o/oo.
In using the regression formula it can be assumed that the residual error is normally distributed with an average equal to 0.
For each analysis result, therefore, ranges (confidence intervals) can be determined, within which the actual b.a.c. of the sample of blood will lie with a given degree of accuracy.
For this purpose, it is necessary to have the s.d. and a table with normal probabilities (which can be found at the back of nearly all books on statistics).

Example: certainty 99%: $\hat{y} + 2.58$ s.d. $> y > \hat{y} - 2.58$ s.d.

*) The significant level was in all cases taken as $\alpha = 0.10$

Figure 2. The relation between ethanol concentration of a standard solution and ethanol/propanol ratio as int. st. propanol 0.10 °/oo



For analysing blood samples with a b.a.c. over 0.50 o/oo a different internal propanol standard is used. The purpose of using this bigger standard is to prevent increasing inaccuracy if the same standard is used.
Figure 2 (see p. 25) shows a series of measurements made for calibrating the gas chromatograph for this internal propanol standard. For these data, too, the extent of the error in predicting the b.a.c. from the ethanol/propanol ratio was not significantly dependent upon the b.a.c. level. The s.d. is 0.025 o/oo.

Comparison of the s.d. for these data with that for Figure 1 (by means of the F-test) gives a significant difference. Although the individual checks for low and high b.a.c.'s show no correlation between the extent of the error of estimation and the b.a.c. level, it must therefore be assumed from the F-test result that the difference between predicted and actual concentrations increases over a wider range.

3.2. Laboratory experiments: Breath analysis

When the investigations were started, two rather complex breath analysers were commercially available:

(a) the "breathalyzer", Stephenson Corporation (U.S.A.)
(b) the "ethanographe", Lucien Etzlinger, Appareils Electroniques, Switzerland.

Two specimens of each device were extensively tested by the Analytical Centre of the Central Laboratory TNO, Delft.
A description of the tests is given in Appendix 2 (see p.51). The main conclusions are given below.

3.2.1. Calibration

The results of a series of tests with one of the analysers (the "ethanographe") are shown in Figure 3a,b,c,d (see p. 27,28,29,30). A number of standard air/alcohol mixtures were analysed. The y axis represents the "theoretical b.a.c.". This is the b.a.c. calculated

Figure 3a. Relation between "theoretical b.a.c." and analyser reading

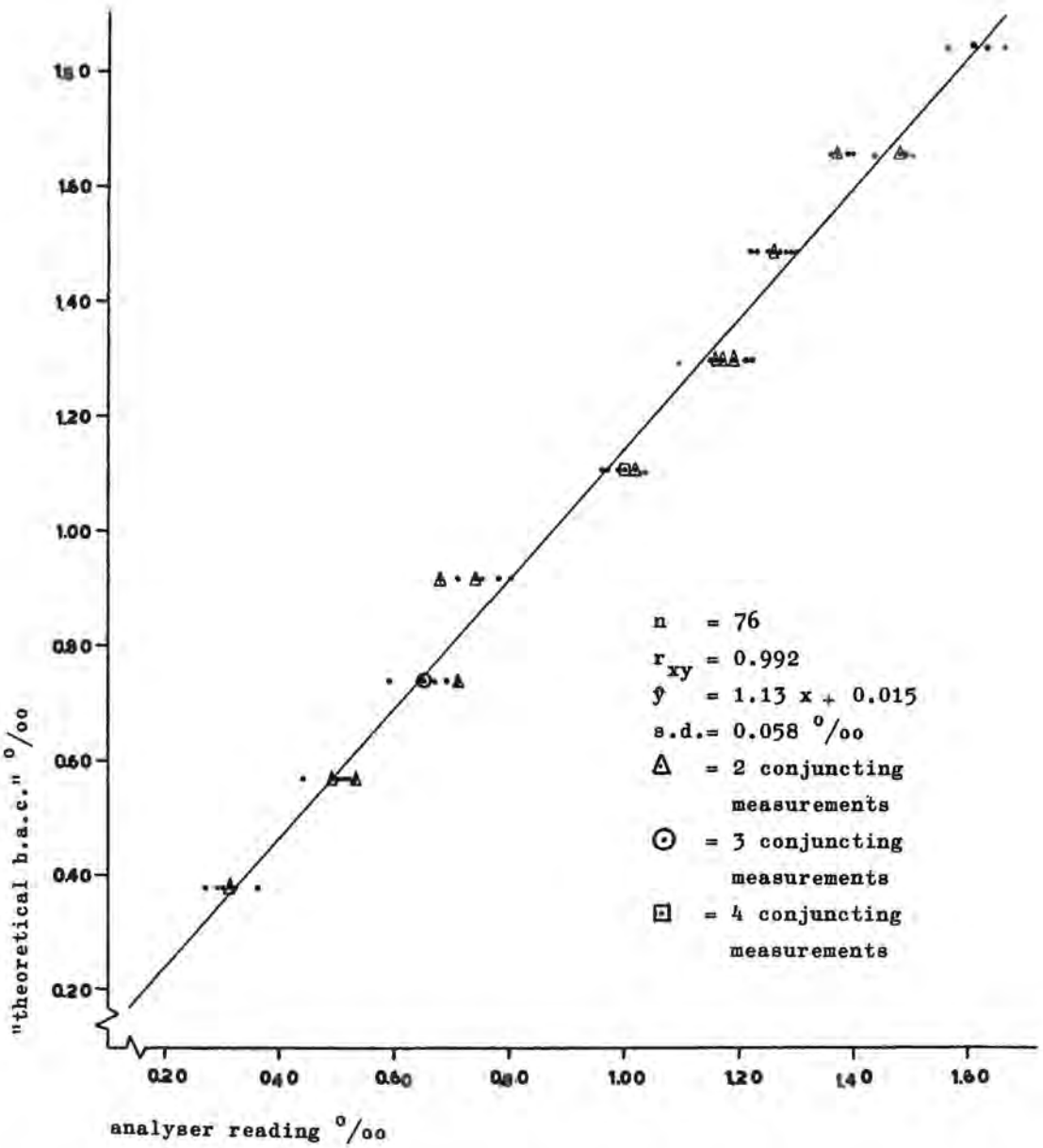


Figure 3b. Relation between "theoretical b.a.c." and analyser reading

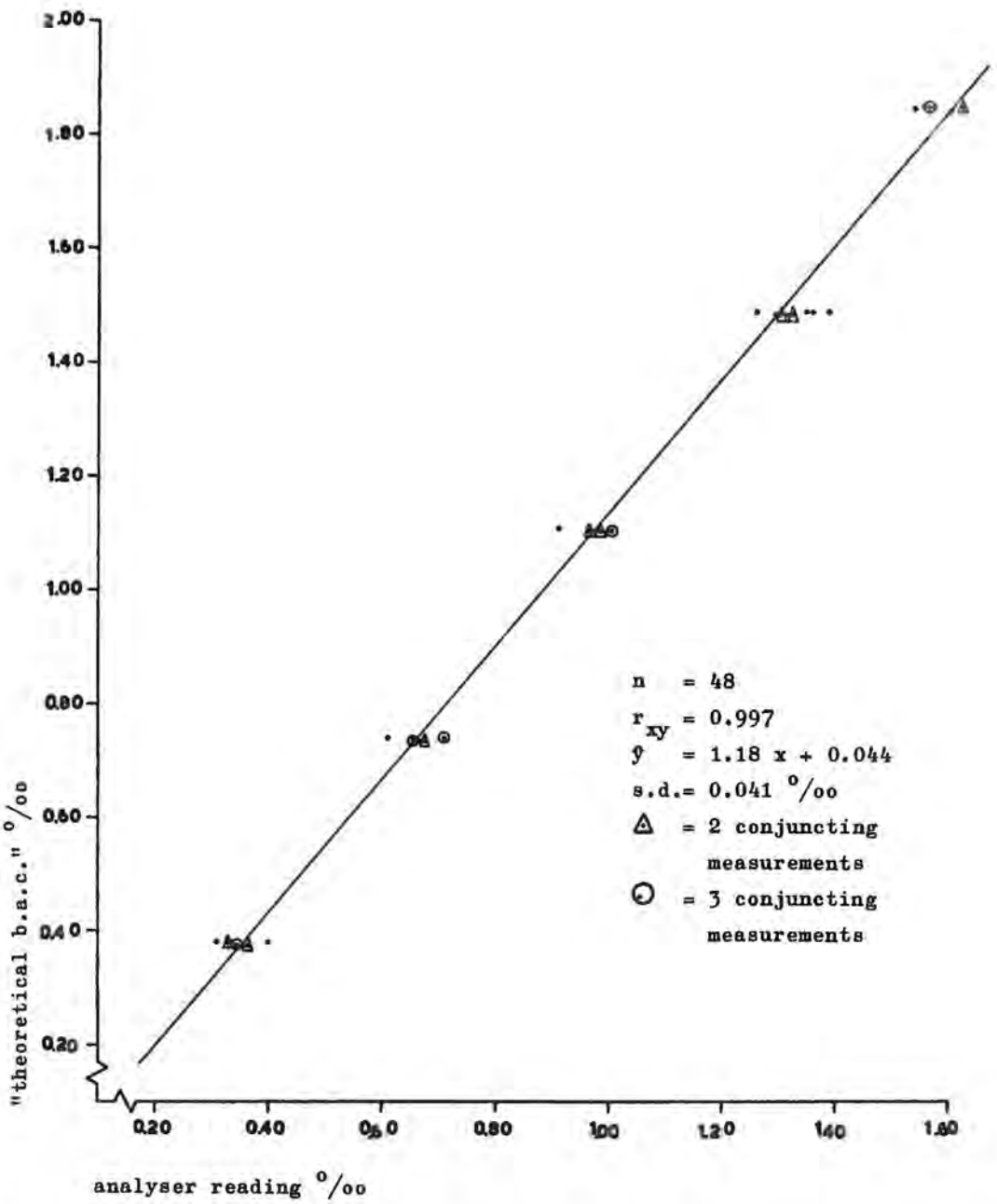


Figure 3c. Relation between "theoretical b.a.c." and analyser reading

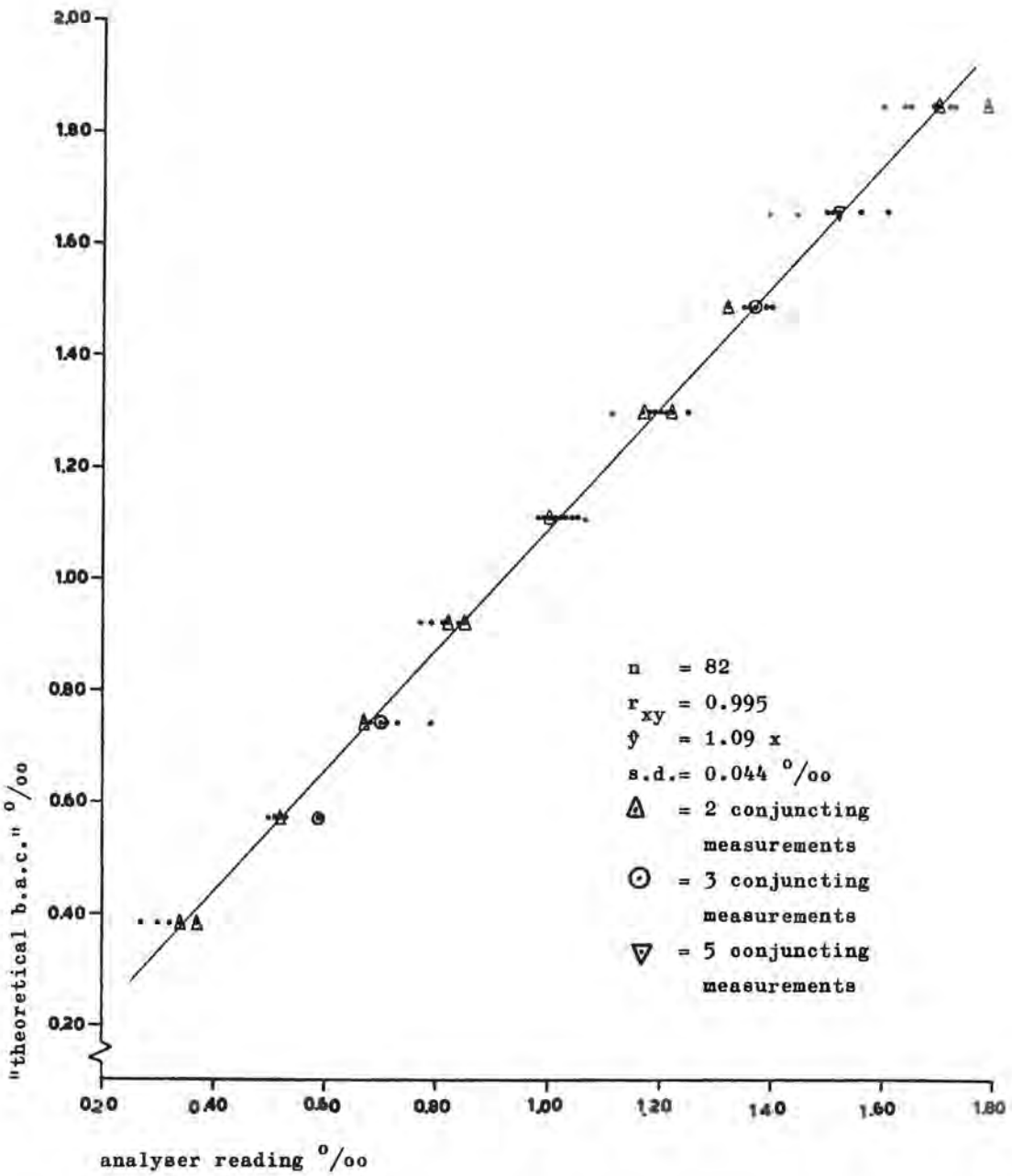
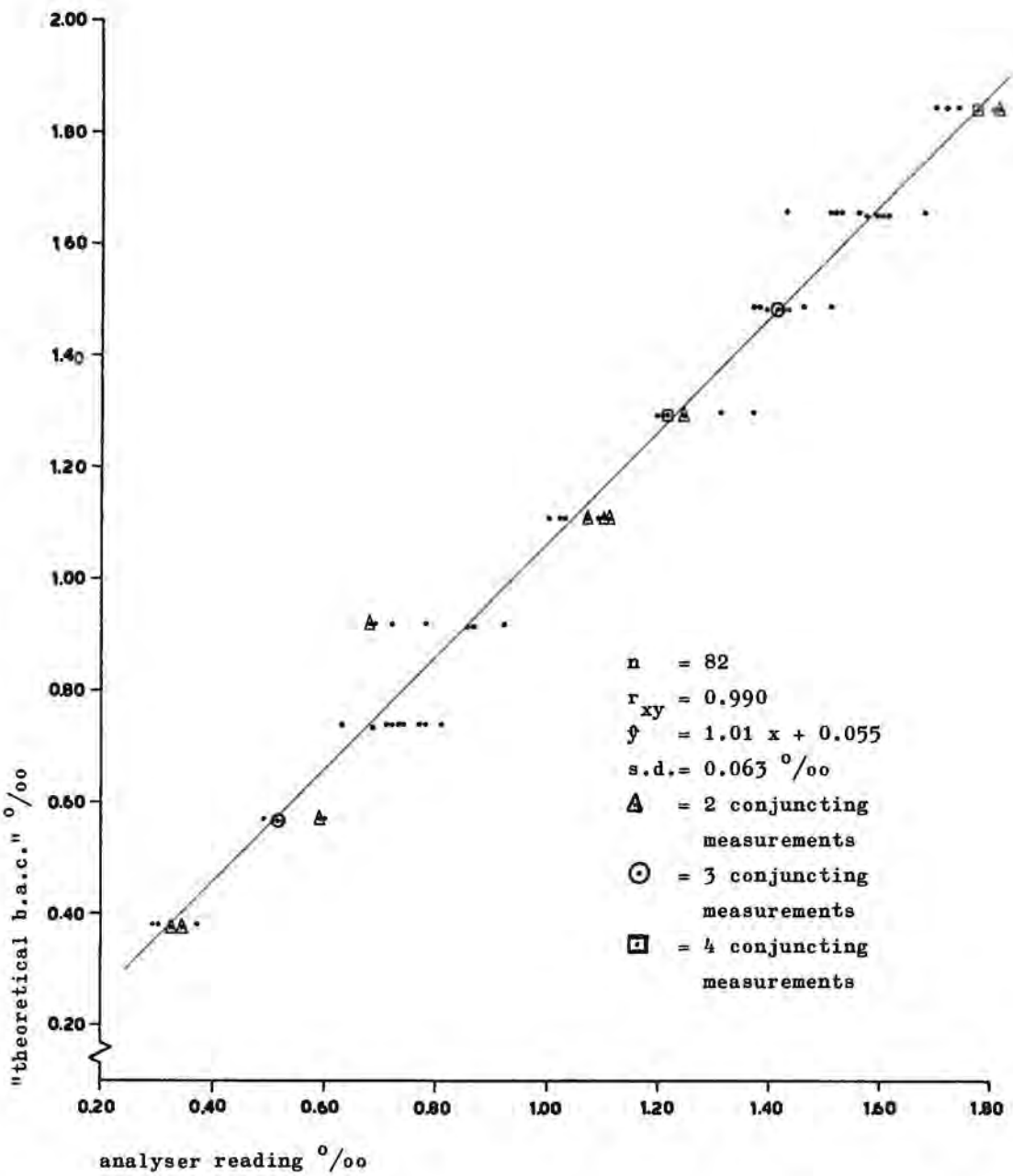


Figure 3d. Relation between "theoretical b.a.c." and analyser reading



with the well-known ratio of 1 : 2100 (between the alcohol content of alveolar breath and that of blood at 34°C), based on the alcohol content of a standard air/alcohol mixture (for details see Appendix 2). The regression formula for predicting the "theoretical b.a.c." from the analyser reading ($\hat{y} = 1.13x + 0.015$) shows that the analyser gives a mean reading about 13% lower than expected from the 1 : 2100 ratio. The three other devices tested also showed this difference, though to a varying extent (See Table 1, p. 48). Possibly the manufacturer calibrates the devices in this way intentionally to avoid persons being accused of having a higher b.a.c. than they really have (Chastain, 1957). But it would be advisable for all devices to be calibrated in the same way, preferably according to the ratio between alcohol in alveolar breath and in blood at 34°C.

3.2.2. Accuracy

In the same way as described in para. 2.1., the "breathalyzer" and "ethanographe" were tested to see whether the difference between theoretical and predicted b.a.c. depended on the b.a.c. level. The result was not significant. For the data in Figure 3, the s.d. of the differences between "theoretical b.a.c." and prediction from the analyser reading is 0.058 o/oo.
Although the differences in accuracy of two devices of the same make proved significant (with the F-test) Table I shows that both makes are equally good.

3.3. Preliminary technological investigations: Description

3.3.1. Time

The investigations were made at night (Friday, Saturday and Sunday nights from about 10.30 p.m. to about 3.30 a.m.) at three successive week-ends in June and July 1968. These times were selected from statistics (Froentjes, 1962; CBS, 1968) indicating that the combination of "drinking and driving" occurred mainly at these times.

3.3.2. Location

The testing vehicle was set up at a different point each night on one of the main roads leading into and out of Middelburg (Sealand). The location was chosen in consultation with the local police.

3.3.3. The testing vehicle

A vehicle suitable for the tests was hired from the Centraal Bureau voor Keuringen op Medisch Hygiënisch Gebied, The Hague.

3.3.4. Procedure

Stopping of Drivers

When everything was ready one of the two police officers present stopped the driver of the first oncoming car or moped.
The police officer told the car driver or moped rider that scientific investigations were being carried out in which he was invited to co-operate voluntarily. For a brief explanation of the investigations the officer referred the driver to an interviewer.
If he agreed to take part, the interviewer took him to the vehicle.

First breath analysis

In the first compartement of the vehicle there was an "ethanographe". This was one of the two tested by the laboratory. The "ethanographe" had been chosen, instead of the "breathalyzers" likewise tested, purely because of the personal preference of the analyst who used the device.
A "breathalyzer" was kept standing by in case any technical defects occured in the "ethanographe". The analyst was employed at the laboratory where the devices had already been tested. He therefore knew how to use the device and how it worked.
The "ethanographe" was supplied by a 220 volt A.C. power unit and was on a sheet of foam plastic on a table. This proved adequate to

prevent galvanometer needle vibrations from interfering with the analysis. The driver was instructed to blow as far as possible into the mouthpiece. The analyst looked to see whether the driver had done so.

Interview

The driver was next taken to a second compartment where the interviewer asked a number of questions about personal characteristics, road experience, driving history, route and alcohol consumed that evening. For this report, the most important information is that about alcohol consumed during the evening and especially whether the driver had drunk any alcohol just beforehand, in which case the breath analysis result might be influenced by alcohol remaining in the mouth.

Bloodsampling

After answering the questions, the driver was asked to go to a third compartment, where a medical doctor questioned him to ascertain any indications against taking a blood sample. In the absence of these, the driver's permission was asked to take a sample of blood. This was done by vein puncture with a disposable needle, the skin first being disinfected with hexachlorophene soap. The driver lay down on an examination table while the sample was taken. To avoid inconveniencing him, the vein was looked for very quickly. If the needle could not be inserted rapidly and painlessly, no further attempt was taken to take a sample.
The volume of blood (about 2 cc) was enough to fill four polypropylene cups. These were coated inside with an anticoagulant (sodium fluoride and potassium oxalate) and could be sealed hermetically after filling.

In the first instance, two of the four cups were to be placed immediately after filling in a refrigerator at 4^{o}C. On the Monday morning after week-end testing these cups were to be put in a bag containing refrigerating elements and taken by car to the laboratory

where they were to be placed in a refrigerator again pending gas chromatographic analysis.
Comparison of the results of analysing two cups stored under refrigeration would also enable the influence of refrigerated storage in these cups to be ascertained.
This sampling, storage and analysis procedure is expected to provide the most accurate determination of "true" b.a.c. obtainable under practical conditions.

It will be clear, however, that it makes no allowance for any differences between arterial and venous b.a.c.'s or for fluctuations in b.a.c. Both occur when the driver is in the absorption stage of alcohol consumption. Arterial blood, however, is difficult to sample and it is asking too much of a volunteer to allow two samples to be taken (with the object of eliminating the effect of fluctuations by averaging the results).

The other two cups were to be kept at the prevailing outside temperature, mailed on Monday morning to the laboratory and cooled there to $4^{\circ}C$.
With this procedure:
(a) the effect of non-refrigerated storage in these cups could be determined;
(b) it could be ascertained whether the effect of non-refrigerated storage in these cups can be compensated by averaging the results for two unrefrigerated samples.

Right after the first week-end, however, the number of blood samples for analysis proved too much for the laboratory's capacity at that time, and it was decided to fill only two cups, and to store one under refrigeration and one not.

Second breath analysis

After the sample of blood had been taken, the driver was asked to blow once more into the ethanographe. This second breath analysis was

for determining the reproducibility of breath analysis. Lastly, he was given a cup of (freshly made) coffee. The entire testing procedure took about fifteen minutes per driver.

Stopping of next driver

Just before the driver reached the second breath analysis, the police officers were told that the next driver could be stopped. Up to midnight there was so much traffic that only drivers from one direction (outgoing traffic) had to be stopped in order to utilise the full capacity of the team of investigators. After midnight, drivers coming from both directions were stopped. The police otherwise occupied themselves in counting the number of cars and mopeds passing by (before midnight outgoing, and after midnight in both directions).

3.4. Preliminary technological investigations: Results

During the time these investigations were made 1947 passing motorists and moped-riders were counted. Of these, 220 were stopped and 175 of them were prepared to go through the complete procedure. Going by the police officers' and interviewer's opinions of their behaviour, those refusing did not decline for fear of discovery that they had been drinking. Nor among those who co-operated did the police find any cases for suspecting an offence against Article 26 of the Road Traffic Act. For each of 157 of the 175 volunteers, two useful breath analysis results were obtained. The reasons for 18 cases not being included were:

(a) the driver had consumed alcohol shortly before: 7 cases;
(b) in the analyst's opinion the driver did not supply a usable breath sample, even after repeated attempts: 5 cases;
(c) faulty operation of the "ethanographe": 2 cases;
(d) technical defect in device: 4 cases.

The results of the blood analysis were available for 147 of the drivers. The reason for 28 cases not being included were:

(a) refusal to allow a sample of blood to be taken: 17 cases;

(b) unsuccessful sampling: 7 cases;
(c) coagulated samples: 3 cases;
(d) incomplete analysis: 1 case.

Ultimately, the result of the second breath analysis and/or the b.a.c. of the refrigerated sample were found to exceed 0.10 o/oo in the case of 39 drivers.

3.4.1. Blood analysis

Gas Chromatography

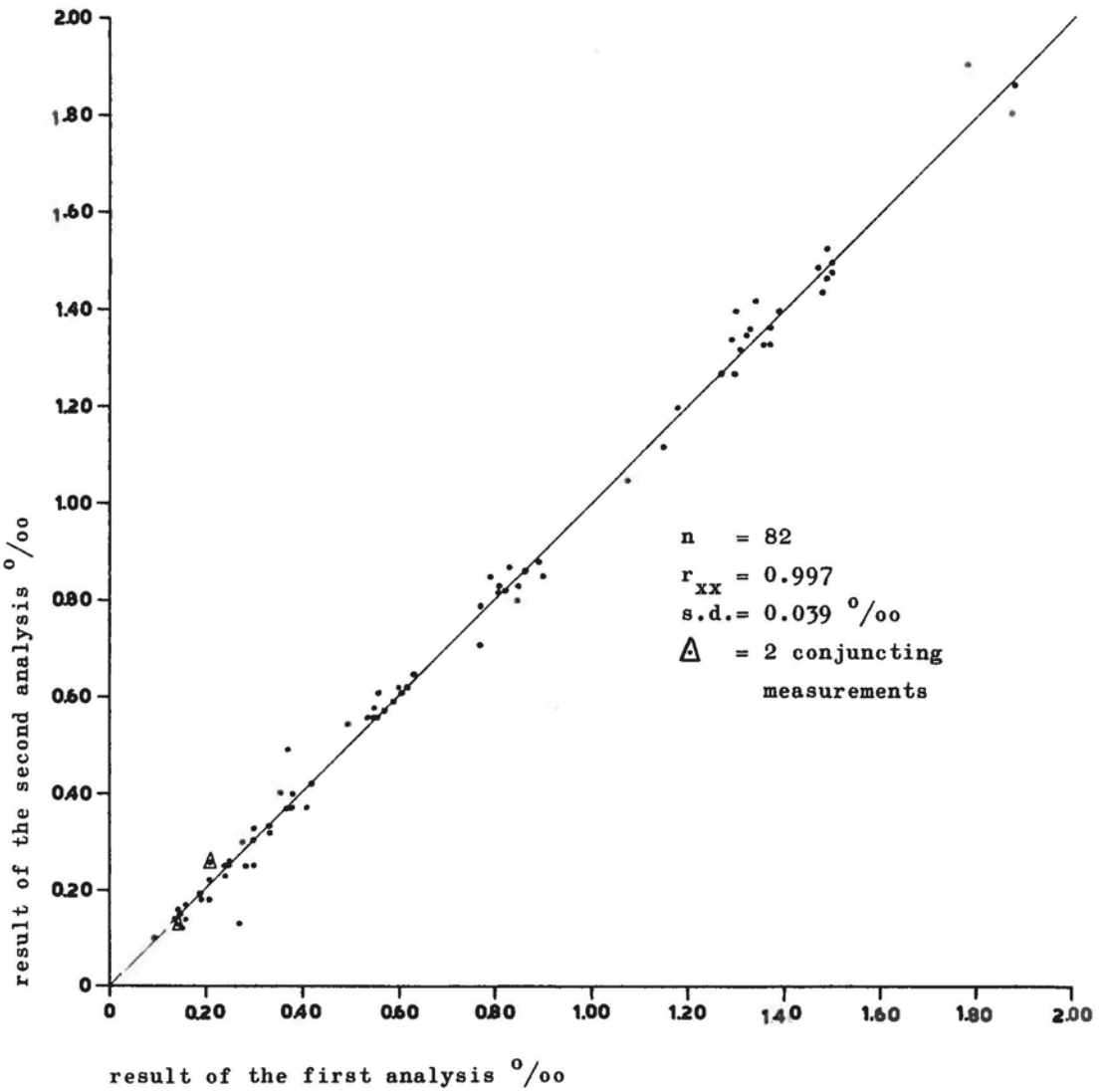
Since the blood from one cup had not been analysed three times but twice in a number of cases, the results of the third analysis were disregarded in further calculations. Comparison of the results of the first and second analyses are given in Figure 4 (see p. 37). This includes only cases (82) in which one or both analyses gave a b.a.c. exceeding 0.10 o/oo. Cases in which both results were below this were of no interest. There is also a possibility that including these cases would distort the picture because there can be no negative values.

As expected, the linear correlation coefficient between both analysis results is very high:

$$r = 0.997.$$

Here again it was checked whether the s.d. was dependent upon the b.a.c. level. This was done by squaring the difference between first and second analyses (d^2) and then correlating it with the closest approximation of the "true" b.a.c., viz. the average of the two results ($\bar{x}$). This $r_{d^2\bar{x}}$ was not significant. For the range over which b.a.c.'s were measured (0.10 - 1.80 o/oo) therefore, there is one s.d. The difference compared with the laboratory experiments (increased difference between predicted and "true" b.a.c.'s over a wider range of alcohol concentrations) is explained by the lack

Figure 4. Relation between results of two analyses by gas chromatography of the same blood sample



of relatively high b.a.c.'s in the samples. The s.d. for the differences between first and second analyses is 0.039 o/oo. The formula for calculating this s.d. is:

$$\text{s.d.} = s_x \sqrt{2(1-r_{xx})} \qquad (2)$$

in which:

s_x is the standard deviation for the result of one analysis in the samples examined

r_{xx} is the linear correlation between the first and the second analyses.

For comparison with the s.d. found in the laboratory experiments, the s.d. for the differences between first and second analyses must not be calculated, but that for those between one analysis and the "true" b.a.c. of the sample. This is because in the laboratory experiments the s.d. was calculated for the differences between the predicted b.a.c. and that of a standard alcohol solution whose exact alcohol concentration is known.

The following formula gives the s.d. if the analysis result is considered to replace the "true" value:

$$\text{s.d.} = s_x \sqrt{1 - r_{xx}} \qquad (3)$$

(A comparison of formula 2 and 3 shows that the s.d. for the differences between two analyses is $\sqrt{2}$ times as great as that for the differences between one analysis and the "true" b.a.c. because the "true" b.a.c. cannot be inaccurate).

Application of formula 3 gives s.d. = 0.028 o/oo.

The average of the two analyses is a closer approximation to the "true" b.a.c. of the blood sample than each individual analysis. (Below, therefore, the b.a.c. of a blood sample relates to the average of two analyses). The s.d. for the differences between two averages of two analyses will be less than 0.039 o/oo. This s.d. cannot be calculated exactly because the blood from one and the same

cup would then have to be analysed four times. The value that would then be found, however, can be estimated with the formula:

$$r_{kk} = \frac{K \cdot r_{xx}}{1 + (K-1) r_{xx}} \qquad (4)$$

in which:

r_{kk} is the estimated linear correlation coefficient between two averages of K analyses;

K is the number of analyses averaged;

r_{xx} is the linear correlation coefficient between two analyses.

With formula 2 (in which r_{xx} is replaced by r_{kk}) the s.d. can then be calculated again.

The s.d. for the differences between two averages of two analyses can now be estimated as 0.023 o/oo.

The s.d. for the differences between the average of two analyses and the "true" b.a.c. is, according to formula 3, then s.d. = 0.016 o/oo.

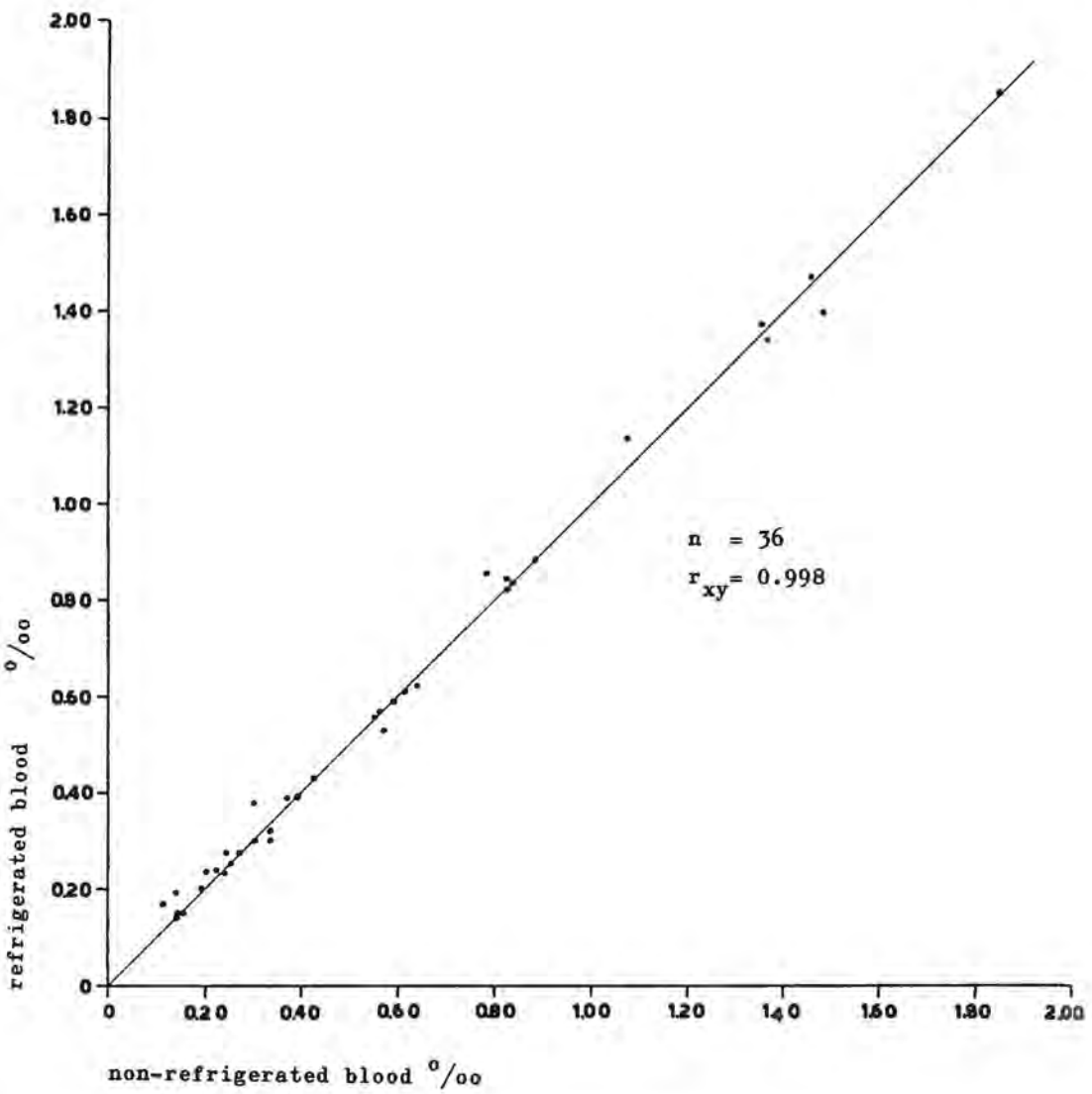
The s.d. for a single analysis (0.028 o/oo) is indeed greater than the s.d.'s found in the laboratory experiments (0.025 and 0.011 o/oo) but this can be compensated by repeating the analysis. By taking the average of two (or more) analyses, the accuracy of routine determinations (0.016 o/oo) does not differ materially from that of a single analysis of a standard sample.

Storage and transport of blood samples

It was found after analysis of the samples that in 36 cases the refrigerated and/or non-refrigerated sample showed a b.a.c. greater than 0.10 o/oo. (average of two analyses). The b.a.c.'s of both samples in these cases can be compared by reference to Figure 5 (see p.40).

The linear correlation coefficient between both variables is r = 0.998. The relationship between the s.d. and the b.a.c. level was again checked as follows. The b.a.c. of the refrigerated sample was

Figure 5. Relation between b.a.c. of refrigerated and of non-refrigerated blood



predicted from the b.a.c. of the non-refrigerated sample with the aid of a linear regression formula. The difference between predicted and actual values was squared and correlated with the actual value. The $r_d^2{}_y$ that was found, 0.252 is low but nevertheless deviates significantly from 0. No s.d. can therefore be determined which applies to the entire range of b.a.c.'s found. The number of observations is too limited, however, for calculating separate s.d.'s for successive b.a.c. intervals. When two analyses of blood from the same cup were compared this phenomenon (increase in s.d. with higher b.a.c.) did not occur. It can consequently be concluded that the (probably slight) effect of storage and/or non-refrigeration will play a part in the case of high b.a.c.'s. The question which of the two factors ("storage" or "non-refrigeration") has more effect cannot be answered directly. The b.a.c. of a second refrigerated sample (and if possible a second non-refrigerated sample) would have to be available. The question is answered indirectly, however, in para. 3.4.3. As relatively high b.a.c.'s in fact hardly occur among the samples, it cannot yet be said with certainty whether samples should be refrigerated immediately.

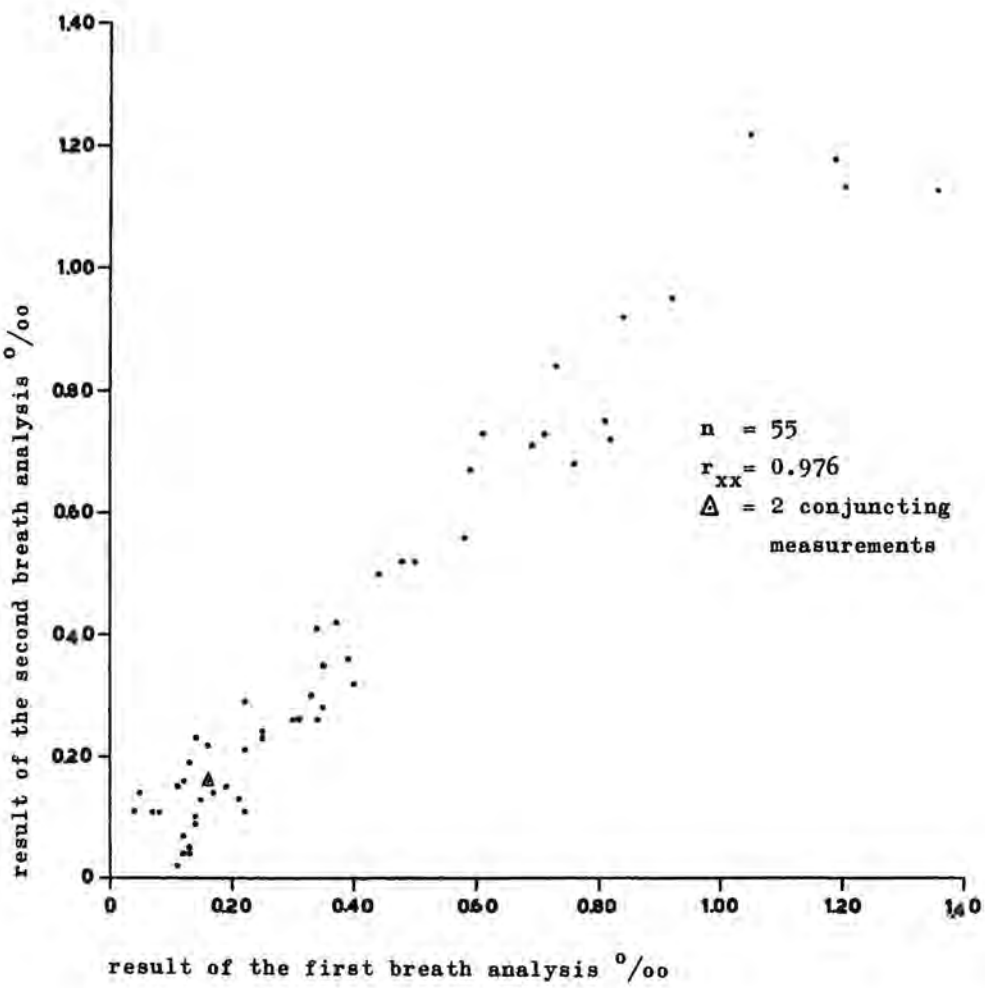
3.4.2. Breath analysis

The results of the first and second analyses are compared in Figure 6 (see p.42).
There were 55 cases in which the first and/or second result exceeded 0.10 o/oo. The linear correlation coefficient between the two results was 0.976. The s.d. proved to depend on the b.a.c. level ($r_d^2{}_{\bar{x}} = 0.451$) and here again, therefore, no s.d. could be calculated. This was not found to be the case in the laboratory experiments with the ethanographe and is therefore due to the conditions of the preliminary technological investigations. These data are interpreted further in para. 3.4.3.

3.4.3. Comparison of blood and breath analyses

In describing the investigations (para. 3.3.) it was stated the the b.a.c. of the refrigerated sample was expected to be the most accurate

Figure 6. Relation between the results of the first and second breath analyses



determination of the "true" b.a.c. attainable under practical conditions. The breath analyses must therefore be compared with the b.a.c. of the refrigerated sample. For comparison of blood and breath analyses, 39 cases are available in which the breath analysis and/or the b.a.c. of the refrigerated sample exceeded 0.10 o/oo.

Change in b.a.c. between the two breath analyses

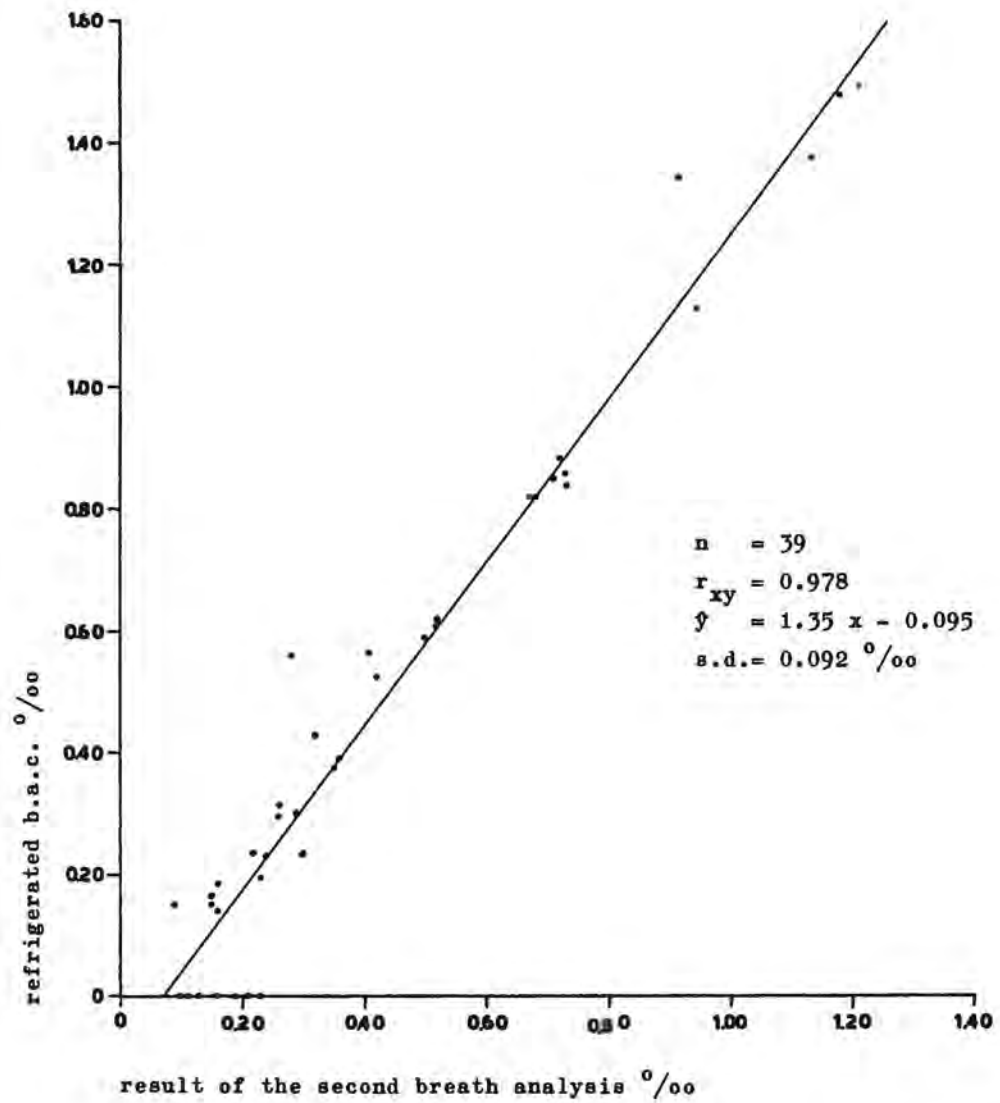
Owing to the test procedure, the first breath analysis was made 10 to 15 minutes before the sample of blood was taken and the second breath analysis was several minutes after blood sampling. If the driver's "true" b.a.c. does not change between the two breath analyses, both should have the same value for predicting the b.a.c. of the refrigerated sample than either of them alone. All this can be checked by comparing the linear correlation coefficient between b.a.c. of the refrigerated sample and the first breath analysis ($r_1 = 0.965$), the second breath analysis ($r_2 = 0.978$) and the average of the two ($r_3 = 0.977$), respectively. The difference between r_1 and r_2 does not point to greater value for the second breath analysis, but is not statistically significant. Comparison of r_2 and r_3, however, shows that averaging the two breath analyses gives no gain over the second analysis alone in predicting the b.a.c of the refrigerated sample. This suggests that there is a change in the driver's "true" b.a.c. between the two breath analyses.

Figure 6 indicates that this change involves both an increase (in the absorption stage) and a decrease (in the elimination stage). This interpretation is substantiated by the non-significant result of a non-parametric sign-test. Consequently, these investigations cannot determine the reproducibility of the breath analysis. For this purpose two breath analyses will have to be made in direct succession.

Calibration of breath analysis

For certainty, a more detailed comparison was made between blood and breath analysis based on the result of the second breath analysis. The second breath analysis and the b.a.c. of the refrigerated sample are compared in Figure 7 (see p.44).

Figure 7. Relation between b.a.c. of refrigerated blood and the result of the second breath analysis



It was again checked whether the difference between predicted and actual b.a.c. of the refrigerated blood depended upon the b.a.c. level. This was not the case, and the linear regression formula can be used for predicting the b.a.c. of the refrigerated sample from the second breath analysis. This formula is:

$$\hat{y} = 1.35\ x - 0.095$$

in which $\hat{y}$ is the predicted b.a.c. of the refrigerated sample and x is the result of the second breath analysis.
The original calibration of the ethanographe used for the investigations is given in para. 3.2.2. ("ethanographe" 1). With this original calibration and the regression formula found in the preliminary technological investigations, the correct calibration of the device can be determined. The ratio for alcohol content of exhaled air and the b.a.c. (which has to be used for calibrating the ethanographe) will be smaller than the 1 : 2100, which is based on theoretical considerations.

Accuracy of breath analysis

The s.d. of the differences between predicted and actual b.a.c. of the refrigerated sample is 0.092 o/oo. These differences cannot be attributed solely to breath analysis. The accuracy of determining the b.a.c. by blood analysis also plays a part. Sources of error that may affect this are metioned in para. 2.1.: origin of the sample, fluctuations in b.a.c. during absorption, storage of samples and method of analysis.
It has already been pointed out that some drivers are likely to be in the absorption stage. The first two sources of error cannot therefore be disregarded in this case. As regards storage and/or non-refrigeration of samples it was said that the (probably slight) effect of this will play a role at high b.a.c.'s. The inaccuracy of the analysis method is the only source of error expressable numerically. Para. 3.4.1. shows that the influence of the blood analysis method itself on the differences between predicted and actual b.a.c. of the refrigerated sample is in fact very slight.

Lastly, it must be remembered that it is possible to use the average result of two breath analyses made in direct succession. This will present a gain in predicting the b.a.c. of the refrigerated sample.

Increasing differences at higher b.a.c.'s

The difference between predicted b.a.c. of the refrigerated sample based on the second breath analysis and the actual b.a.c. of the refrigerated sample does not increase at higher b.a.c.'s. It can thus be assumed that the accuracy with which the two compared variables (second breath analysis and b.a.c. of the refrigerated sample) are determined is independent of the b.a.c. level. An apparent contradiction of this assumption is that the difference between first and second breath analyses and that between b.a.c. of the refrigerated and unrefrigerated sample does increase with the b.a.c. level. This contradiction disappears, however, if the following assumptions are made:

(1) The increase in the difference between the first and second breath analyses is not consequent upon the accuracy of breath analysis, but upon changes in the "true" b.a.c. between the two analyses, which becomes greater at higher b.a.c.'s.

(2) The increasing difference between b.a.c. of the refrigerated and non-refrigerated sample at higher b.a.c.'s is not due to storing the samples in the polypropylene cups, but to non-refrigeration of the second sample, which will have an effect at high b.a.c.'s.

3.5. Conclusions

The results of SWOV's research so far can be summarised as follows:

(1) The accuracy of a single gas chromatographic analysis of standard alcohol solutions can be indicated with an s.d. = 0.011 o/oo for concentrations below 0.50 o/oo, and with an s.d. = 0.025 o/oo for higher concentrations.

(2) Breath analysers are calibrated too low by their manufacturers. Their accuracy when analysing standard air/alcohol mixtures is indicated by an s.d. of 0.040 to 0.065 o/oo.

(3) The s.d. of gas chromatographic analysis of blood samples is estimated at 0.016 o/oo when averaged over two analyses.
(4) It is assumed that storage of blood samples in polypropylene cups does not influence the accuracy of the analysis method. If the samples are not refrigerated forthwith, its accuracy is likely to decrease at higher b.a.c.'s.
(5) The drivers' "true" b.a.c. may either increase or decrease during the test which lasts about 15 minutes. These changes probably also increase at higher b.a.c.'s.
(6) Reproducibility of breath analysis could not be confirmed.
(7) Calibration of breath analysers, such as the "ethanographe", will have to be based on a ratio for the alcohol content of exhaled air, and the b.a.c. lower than the 1 : 2100 based on theoretical considerations.
(8) The accuracy of the predicted b.a.c. based on breath analysis is represented by an s.d. of 0.092 o/oo. It is not yet known how much can be gained by using the average of two breath analyses in direct succession.
(9) In view of the nature of the preliminary technological investigations in which they were arrived at, 4, 5, 7, and 8 must be treated as hypotheses which require checking during this more extensive research.
(10) As compared with the preliminary investigations, this more extensive research should be modified as follows:
(a) a larger collection of samples, including higher b.a.c.'s.
(b) a second sample of blood stored under refrigeration and perhaps a second sample stored without refrigeration.
(c) two breath analyses made in direct succession shortly before or after the sample of blood is taken.
(d) fairly accurate determination of the stage of the driver's alcohol consumption (for instance by means of two breath analyses with at least 15 minutes in between).
(e) breath analysis with the aid of one of the recently developed devices.

Table 1. Calibration and accuracy of tested breath analysers

device	regression formula 1)	s.d. °/oo	n
ethanographe 1	$\hat{y} = 1.13\ x + 0.015$	0.058	76
ethanographe 2	$\hat{y} = 1.18\ x - 0.044$	0.041	48
breathalyzer 1	$\hat{y} = 1.09\ x$	0.044	82
breathalyzer 2	$\hat{y} = 1.01\ x + 0.055$	0.063	82

1)
$\hat{y}$ is predicted "theoretical b.a.c."
x is analyser reading

mg ethanol/2 l water	mg ethanol/litre air	°/oo ethanol in blood (theoretical b.a.c.)
900	0.18	0.37
1350	0.27	0.56
1800	0.35	0.74
2250	0.44	0.93
2700	0.53	1.11
3150	0.62	1.30
3600	0.71	1.48
4050	0.79	1.67
4500	0.88	1.85

Table 2. Ratio ethanol in water, ethanol in air (breath), ethanol in blood

APPENDIX 1

ETHANOL DETERMINATION WITH GAS CHROMATOGRAPHY 1)

Instrument : Pye gas chromatography, Model 104, with flame ionisation system 2)
Column : Glass column of diatomite C with 10% polyethylene glycol 400, 100-120 mesh, length 5 feet
Temperature: 85° C
Carrier gas: N_2, pressure 1.2 kg/cm^2; 150 ml per min.
Gas : H_2, pressure 1.4 kg/cm^2; 100 ml per min.
air, pressure 1.3 kg/cm^2; 700 ml per min.
Diluter : Griffine Diluspence type 221.

Determination procedure:

20 µl blood is diluted with 200 µl of a propanol solution in water (25 mg propanol in 100 ml distilled water for concentrations over 50 mg%EtOH; 10 mg propanol in 100 ml water for concentrations below 50 mg%EtOH). When the gas chromatograph is ready for use, 1 µl of the diluted blood is injected with an SGE injector. The ethanol concentration can be calculated from the ratio between the ethanol and propanol peaks.

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1) These data were furnished by Mrs.Dr. H.J. Leijnse-Ybema of the Central Laboratory for Clinical Chemistry of Dijkzigt Hospital, Rotterdam.
2) The gas chromatograph was kindly lent by N.V. Philips Gloeilampenfabrieken, Eindhoven

Calibration of the gas chromatograph:

Instead of a sample of blood, ethanol solutions of known strength were diluted as described above and determined with the gas chromatograph. The gas chromatograph is calibrated quarterly, in order to check any movements in the figures. For day-to-day alcohol determinations, one or two standards are always taken for verification.

APPENDIX 2

REPORT ON LABORATORY EXPERIMENTS WITH BREATH ANALYSERS 1)

1. INTRODUCTORY

The following were available for testing as devices for determining the blood alcohol concentration by analysis of exhaled air. (These devices will be called "analysers").
Two breathalyzers (2788 and 2787) made by Stephenson Corporation;
Two ethanographes (CV 588 and CA 700) 2) made by Lucien Etzlinger, Appareils électroniques.
The diagrams in Figures 8 (p. 52) and 9 (p. 53) show how the analysers work. The purpose of the test was to assess the calibration and accuracy of the analysers with the aid of ethanol/air mixtures.

---

1) Abstract from report CL 69/91 by Central Laboratory TNO-Delft, P. Slingerland and Dr. G.J. van Kolmeschate.

2) Ethanographe CA 700 was lent by H.Th.P. Cremers, police surgeon, Rotterdam, ethanographe CV 588 by Lucien Etzlinger, Geneva.

Figure 8. Diagram of the "breathalyzer"

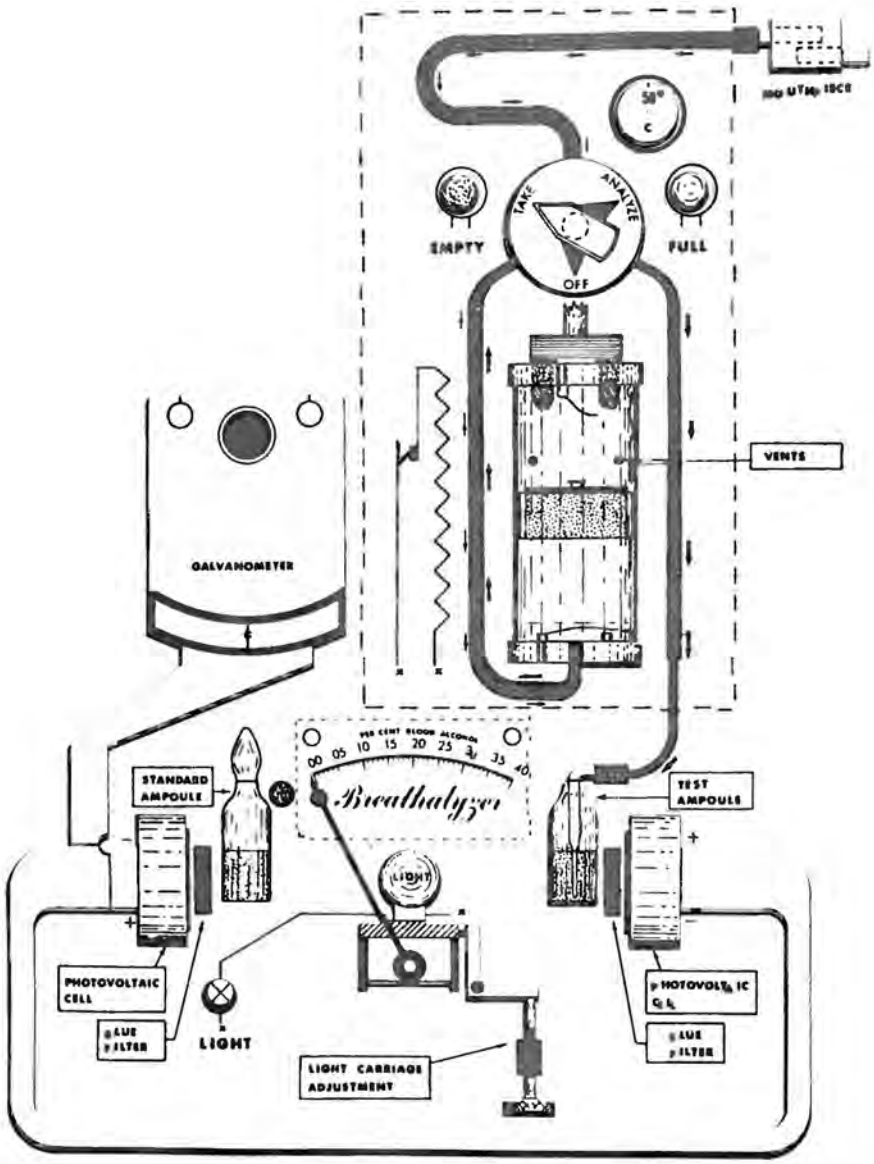
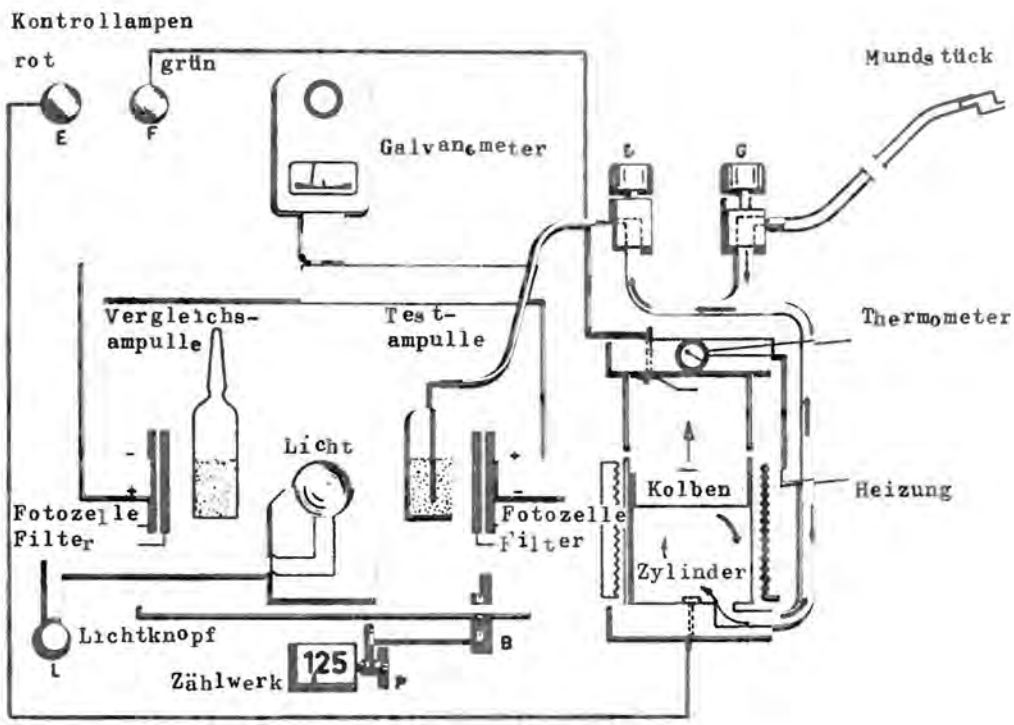


Figure 9. Diagram of the "ethanographe"



2. TESTS

2.1. Equipment

The sampling device consists of a washing bottle (with an inlet tube provided with a sheet of sintered glass), containing a water/ethanol mixture. This bottle is placed in a water-bath thermostat, 34.0 ± 0.1°C; 34°C was taken, as being the general average temperature of the breath leaving the mouth. The bottle inlet is connected with the compressed air tube via a volume meter. The outlet consists of a glass tube with a plug of cotton wool heated with an infra-red lamp to prevent condensation. The plug is to retain any extraneous particles.

2.2. Water/ethanol mixtures

Production of a series of air/ethanol mixtures with the equipment described in this Appendix 2, para 2.1. was based on a series of standard mixtures of water and ethanol.
The solutions were prepared by carefully weighing a quantity of absolute ethanol and making it up to two litres with distilled water.

2.3. Air/ethanol mixtures

The relationship between the ethanol concentration in water and the relevant ethanol concentration in the air passed through is generally indicated as a quotient Q:

$$Q = \frac{\text{mg ethanol per litre liquid}}{\text{mg ethanol per litre air}}$$

Q depends upon the temperature and composition of the liquid being examined.
Grosskopf determines $Q_{34^\circ} = 2550$ for pure water.
Borkenstein gives in the "breathalyzer" Instruction Manual:

$$Q_{34^\circ} = \frac{60.5 \times 10 \times 2 \text{ (mg ethanol per litre water)}}{\frac{1.0}{2.1} \text{ (mg ethanol per litre air)}} = 2541$$

The difference between these two figures is negligible.
In the calculations Q was taken as 2550. Grosskopf does not indicate the accuracy of his measurements. He does conclude that the Q values in the literature do not correspond, owing to systematic errors made in determining low concentrations of ethanol.
The volume of ethanol in the air/ethanol mixture is thus calculated as follows:

a mg ethanol per 2 litres water $\longrightarrow \frac{1}{2}$ a . $\frac{1}{2250}$ mg ethanol per litre air.

Furthermore the appropriate °/oo ethanol in blood was calculated from the formula (see Borkenstein, 1963, p. 12):

$$Q = 2100 = \frac{\text{mg ethanol per litre blood}}{\text{mg ethanol per litre air}}$$

These data are compiled in Table 2 (see p. 48).

2.4. Accuracy of air/ethanol mixtures

In earlier tests made at the Analytical Centre of the Central Laboratory TNO-Delft, at the beginning of 1967, several Q values were checked by gas chromatography. The conclusion was that the order of magnitude was good. This method of checking, however, is too rough for determining the accuracy of the ethanol concentration in a current of gas as the basis for verifying the devices under test. A suitable analysis method

was sought for determining the ethanol concentrations, as the results of such tests are indispensable for ascertaining the absolute accuracy of the breath analysers.

The chemical determination of low ethanol concentrations is unlikely to be more accurate than the chemical determination method of the breath analysers under test. Moreover, it is very laborious.

Examination with an infra-red-spectrophotometer showed the lower limit of determination to be at 5 mg ethanol/litre N_2 (CL-TNO, undated), while our upper limit is 1 mg ethanol/litre air.

The expectation that determination might be successful with an infra-red gas analyser (a non-dispersive I.R. spectrophotometer) was confirmed by Payne et al. (1966).
The infra-red analyser used for ethanol vapour has a full-scale sensitivity of 400 ppm (v/v) ethanol vapour (≃ 0.73 mg/l air at 34°C and 76 cm Hg); the reading is lineair over the range from 0 - 400 ppm. Based on a cylinder mixture of 300 ppm ethanol in air (≃ 0.55 mg/l air at 34°C) he found a range of 6 ppm. He speaks of 'successive readings" and does not state the number of observations. If he made four observations, the standard deviation would be 3 ppm (≃ 0.06 mg/l air at 34°).
As the Analytical Centre does not posses an I.R. gas analyser, it was not possible to make such an analysis.

2.5. Procedure

The compressed air was blown in at a velocity of 3 l/min, the quantity being 1/2 - 1 l. In operating the analysers, the instructions for using the "breathalyzer" and the "ethanographe" were followed. With both types, a waiting time of 1 1/2 minutes was observed after the air for testing had flowed into the ampoule ("ethanographe" instructions give 1 minute).

3. RESULTS

The results are given in Figures 3a,b,c,d (see p. 27-30) and in Table 1 (see p. 48). The number of observations differ from mixture to mixture, depending solely on working conditions. It must be stressed that all observations were noted, except for the first 6 out of the 10 when testing ethanographe CV 588 with a mixture of 0.88 mg ethanol/litre air; it was found that owing to the analyser tube being twisted the observations had varies greatly and were too low. After this had been remedied, another 4 observations were made.
Owing to a shortage of ampoules for the "ethanographe", only 5 series of observations were made with CA 700.
A new ampoule was used for each observation.

3.1. Calibration

Table 1 shows the linear regression formula for predicting the "theoretical b.a.c." from the analyser readings.
The linear regression curve is the best calibration curve (the average difference between "theoretical" and predicted b.a.c. equals 0) conditional upon the accuracy of the prediction being independent of the b.a.c. level. This was checked for ethanographe CV 588 and breathalyzer 2788 by squaring the differences and then correlating them with the "theoretical b.a.c.". Neither correlation coefficient showed a significant deviation from 0. It was consequently assumed that the condition for using the linear regression formula was satisfied. It can be seen from the formulae that all four analysers give lower average than the "theoretical b.a.c." But the four analysers do this to different degrees.

3.2. Accuracy

The accuracy of the analysers is indicated by the standard deviation (s.d.) between "theoretical" and predicted b.a.c.'s.
On the basis of the values given in Table 1 it can be stated that breath analysers such as the "ethanographe" and the "breathalyzer" have an s.d. of 0.040 to 0.065 °/oo.

The accuracy with which the air/ethanol mixtures were composed is not known (see para 2.4.).
It is not certain, therefore, whether the differences found between "theoretical b.a.c." and the prediction thereof (based on the analyser reading with the aid of the regression formula) are attributable solely to the breath analysers. The difference in accuracy between two analysers of the same type was checked with the F-test. The results proved to be significant.
It is however clear that there are no differences as between the two types.

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